Tissue oxygenation response to systemic and localised hypoxia during intermittent isometric contractions.

Simon Gooch

Tissue oxygenation response to systemic and localised hypoxia during intermittent isometric contractions.

By

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A thesis submitted to the University of Bedfordshire in partial fulfilment of the requirements for the degree of Masters of Science by Research

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Authors Declaration

I declare that this thesis is of entirely my own work. It is being submitted for the degree of Masters of Science by Research at the University of Bedfordshire.

This thesis has never been submitted before for any degree or examination at any other University.

Simon Gooch

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Abbreviations

°C	degrees centigrade
µL	micro litre
µM	micro molar mass
1RM	one repetition maximum
8-OhdG	8-hydroxydeoxyguanosine
Akt-mTOR	protein kinase-B mammalian target of rapamycin
ATP	adenosine triphosphate
BFR	blood flow restriction
BLa^-	blood lactate
c	concentration of chromophore
Ca^{2+}	calcium ions
CAT	catalase
CK	creatine kinase
CI	confidence intervals
cm	centimetre
CON	control
CV	coefficient of variation
DPF	differential path-length factor and exit
DTNB	5,5'-dithio-bis2-nitrobenzoic acid
EMG	electromyographic
F_IO_2	fraction of inspired oxygen
G	gauge
GH	growth hormone
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
GSSG	oxidised glutathione
GSSG:GTSH	glutathione ratio
H^+	hydrogen ions
H_2O_2	hydrogen peroxide
Hb	haemoglobin
HHb	de-oxygenated haemoglobin
HIF-1	hypoxic-inducible factor 1
HYP	systemic hypoxia
IGF-1	insulin-like growth factor-1
kg	kilogram
LH	lipid hypoperoxides
M	molar mass
MAPK	mitogen-activated protein kinase
Mb	myoglobin
mCSA	muscle cross sectional area
MDA	malonadialdehyde equivalents
MPS	muscle protein synthesis
mm	millimetre
mmHg	millimetre of mercury
mTOR	mammalian target of rapamycin
MVC	maximal voluntary contractions

NIRS	near-infrared spectroscopy
nm	nanometre
NO	nitric oxide
·O	singlet oxygen
·OH	hydroxyl radicals
$O_2^{\cdot -}$	superoxide radicals
O_2	oxygen
O_2Hb	oxygenated haemoglobin
OD	optical density
p	probability value
p70 S6K	70kDa ribosomal protein S6 kinase
P/A ratio	pro-oxidant/antioxidant balance
PAR-Q	physical activity readiness questionnaire
PC	protein carbonyl
PCr	phosphocreatine
PGC-1α	peroxisome proliferator-activated receptor-y co-activator-1α
pmol	picomole
PP	perceived pain
R^2	coefficient of determination
RE	resistance exercise
reps	repetitions
ROS	reactive oxygen species
RPE	rate of perceived exertion
rpm	rotations per minute
SD	standard deviation
SOD	superoxide dismutase
SpO_2	peripheral capillary oxygen saturation
TAC	total antioxidant capacity
TBARS	lipid peroxidation products
TE	typical error
THb	total haemoglobin
TGSH	total glutathione
TNB	5-thio-2-nitrobenzoic acid
TSI	total saturation index
VAS	visual analogue scale
wk	week
yr	year
XO	xanthine oxidase
XDH	xanthine dehydrogenase

Abstract

The use of either blood flow restriction (BFR) and systemic hypoxia (HYP) during resistance exercise has been shown to increase hypertrophy and strength to a greater extent compared to traditional resistance exercise alone. However, the mechanisms underpinning these enhanced adaptations remain to be elucidated. Differences in skeletal muscle oxygenation may be one of several factors that leads to increased hypertrophy and strength with BFR and HYP. Nevertheless, this has been sparsely investigated. High intensity resistance exercise is also accompanied by an increase in oxidative stress, providing beneficial hypertrophic signalling. The addition of BFR has been observed to decrease these beneficial signals and the effect of HYP is unknown. **PURPOSE:** To investigate the skeletal muscle oxygenation and oxidative stress response during moderate intensity rhythmic isometric handgrip exercise with BFR, HYP, and resistance exercise alone. In addition, to observe the perceived pain (PP) response to these novel exercise modalities during exercise. **METHODS:** Eight recreationally active males (23 ± 1 yr, 76 ± 10 kg, 175 ± 6 cm) completed three sets of 45 repetitions of isometric handgrip exercise (60% 1RM) either with BFR (80 mmHg proximal cuff) 5 minutes pre and during exercise, with HYP (14% O_2) 5 minutes pre and during exercise or with resistance exercise alone (CON). Exercise was completed in a supine position, with one-minute rest in-between sets. Skeletal muscle oxygenation was measured throughout using a dual wave near infrared spectroscopy (NIRS) device placed on the forearm flexors, with output variables of tissue saturation index (TSI), oxygenated haemoglobin (O_2Hb), de-oxygenated haemoglobin (HHb) and total haemoglobin (THb). NIRS variables were reported as a delta from a pre-exercise control period and represented a change from baseline. Oxidative stress was measured in whole blood via glutathione ratio (GSSG:GTSH). PP was measured during each exercise set with a visual analogue scale. **RESULTS:** TSI was lower in BFR (-11.5 ± 10.3 %) compared to CON (-1.3 ± 5.1%, p = 0.007) and lower with no significant difference compared to HYP (-4.5 ± 5.1 %, p = 0.059), there was no difference between CON and HYP (p > 0.05). There was no difference in O_2Hb between conditions (p >0.84). HHb was higher in BFR (13.9 ± 5.1 µmol) compared to both CON (2.06 ± 5.87 µmol, p = 0.001) and HYP (6.83 ± 6.11 µmol, p = 0.042), with no difference between CON

and HYP ($p > 0.05$). THb was significantly higher in BFR (9.41 ± 9.54 µmol) compared to both CON (-1.22 ± 5.50 µmol, $p = 0.001$) and HYP (1.59 ± 5.04 µmol, $p = 0.008$), with no difference between CON and HYP ($p > 0.05$). There was no increase in GSSG:GTSH pre-post exercise with no significant difference between conditions ($p > 0.085$). PP was higher in the BFR condition (6 ± 1 a.u) compared to CON (2 ± 2 a.u, $p = 0.001$) and HYP (2 ± 2 a.u, $p = 0.001$), with no difference between CON and HYP ($p > 0.05$).

CONCLUSION: Moderate intensity rhythmic isometric handgrip exercise with BFR results in an increased blood volume (THb), decreased clearance of HHb and lower TSI compared to HYP and CON, however O_2Hb delivery remains similar between conditions. The differences in skeletal muscle oxygenation with the addition of BFR to resistance exercise provide further insight into the mechanisms acute of BFR; however, further investigation is required with over a prolonged period of training. The current protocol did not elicit a whole blood oxidative stress response in the form of increased GSSG:TGSH, therefore the role of oxidative stress could not be determined.

CHAPTER 1: General Introduction

The maintenance of skeletal muscle mass is of utmost importance to conserve cardiovascular, metabolic and social wellbeing (Pollock et al., 2000). Resistance exercise (RE) is the most potent stimulus to augment skeletal muscle growth (hypertrophy) and strength, however exercise load (intensity) drives this adaptation (McDonagh and Davies, 1984). In untrained humans, increases in muscle hypertrophy and strength generally occur with exposure to an exercise intensity of ≥ 70% of an individual's one repetition maximum (1RM). In response the given stimulus onsets a cascade of biological events, consisting of: metabolic alterations, anabolic hormone secretion, intramuscular signalling and muscle protein synthesis (MPS) (Kraemer and Ratamess, 2005, Scott et al., 2014).

The addition of blood flow restriction (BFR) to the exercising muscle offers a novel alternative, whereby RE training regimes with a low load (20 – 60% 1RM) and BFR have elicited increase in muscular hypertrophy and strength (Abe et al., 2005, Madarame et al., 2008, Yasuda et al., 2010b, Takarada et al., 2000b). The addition of BFR during RE appears to cause no greater risk to safety compared to high intensity RE (Loenneke et al., 2011c); thus providing an alternative to populations who cannot withstand the high mechanical load that high intensity RE elicits on the body, for example the elderly or those rehabilitating injuries. BFR causes a localised low oxygen (O_2) (hypoxic) environment in the exposed limb, resulting in increased metabolic stress through enhanced phosphocreatine (PCr) depletion (Suga et al., 2009, Suga et al., 2012), decreased pH (Suga et al., 2009, Suga et al., 2012) and blood lactate (BLa^-) accumulation (Pierce et al., 2006, Fujita et al., 2007). This enhanced metabolic state caused by BFR up regulates the traditional hypertrophy cascade by favourably modulating: anabolic hormone secretion (Abe et al., 2005, Pierce et al., 2006), intramuscular signalling (Nielsen et al., 2012, Wernbom et al., 2013) and MPS (Fujita et al., 2007, Fry et al., 2010).

Given the aforementioned benefits of RE with a localised hypoxic stimulus (BFR) and the reported physiological benefits of aerobic (Levine and Stray-Gundersen, 2001) and anaerobic (Roels et al., 2007) exercise training in systemic hypoxia (inspiration of low O_2 air) (HYP), investigation has pursued into the potential benefits

of HYP alongside RE (Kon et al., 2012, Kon et al., 2014). In a similar manner to BFR, low intensity RE training in HYP has been shown to increase muscular strength and hypertrophy (Nishimura et al., 2010, Manimmanakorn et al., 2013a, Manimmanakorn et al., 2013b, Kurobe et al., 2014), whilst providing an increased metabolic stress, anabolic hormone secretion (Kon et al., 2010, Kon et al., 2012), intramuscular signalling and MPS (Etheridge et al., 2011), compared to RE in normobaric conditions (sea level).

A decrease in muscle oxygenation is proposed to increase neuromuscular activation and metabolic stress during RE with BFR (Signorile et al., 1991, Takarada et al., 2000a, Takarada et al., 2002, Moritani et al., 1992). Although a hypoxic stimulus is proposed as the major mechanism for the beneficial outcomes of BFR, there has been little investigation into the skeletal muscle O_2 response of this novel modality. Recently, Ganesan et al. (2015) reported that BFR RE (50% 1RM) decreases skeletal tissue O_2 saturation (TSI) significantly during the rest periods between sets (7.5% - 11.2% range), with the contributing factor being an increase in de-oxygenated haemoglobin (HHb). However, during the knee extension exercise itself there was no difference in TSI, although a transient increase in total haemoglobin (THb) was present with BFR as the sets progressed, suggesting venous pooling (Ganesan et al., 2015).

Exposure to systemic HYP during exercise augments a compensatory vasodilation response, whereby the fall in arterial oxygen supply (SpO_2) is matched to preserve muscle O_2 delivery through increased blood flow (Casey and Joyner, 2012). This phenomena has been demonstrated through multiple rhythmic handgrip investigations (Wilkins et al., 2006, Wilkins et al., 2008, Heinonen et al., 2010), with the primarily mechanism being enhanced nitric oxide release from the vascular endothelium (Joyner and Casey, 2014).

High intensity RE has been shown to increase oxidative stress (Steinberg et al., 2002, Lee and Clarkson, 2003, Bloomer et al., 2005, Bloomer et al., 2007). Oxidative stress occurs when the generation of reaction oxygen species (ROS) exceeds the body's ability to neutralise and eliminate these molecules, leading to macromolar damage (Garten et al., 2015). Chronic exposure to oxidative stress can enhance

disease states (Khansari et al., 2009) whereas acute exposures manifested through exercise can increase hypertrophy (Kosmidou et al., 2002, Gomes et al., 2012).

There has been little investigation into the oxidative stress response to RE with either BFR or HYP. Initially, Takarada et al. (2000a) saw no change in the oxidative stress marker lipid peroxide after a 20% 1RM RE session with or without BFR, however there was no high intensity control group to provide a comparison. Goldfarb et al. (2008) went on to discover that moderate intensity RE (70% 1RM) caused a significantly higher increase in oxidative stress markers protein carbonyls (PC) and glutathione ratio (GSSG:TGSH) compared to low intensity RE with BFR (30% 1RM). A recent report has found similar responses, where the addition of BFR to low intensity RE (30% 1RM) blunted the oxidative stress response (PC), however the addition of BFR to moderate intensity RE (70% 1RM) enhanced it (PC, GSSG:TGSH) (Garten et al., 2015). The same group examined BFR with the absence of exercise, both PC and GSSG:TGSH increased significantly with the author suggesting that ischemic reperfusion and xanthine oxidase being responsible for the enhanced oxidative stress response.

It is reasonable to presume that although both BFR and HYP RE have been shown to provide similar benefits to skeletal muscle (increased hypertrophy/strength); these benefits may arise through different mechanisms. Therefore, this study intends to further investigate both the skeletal muscle oxygenation and oxidative stress response to BFR and HYP RE. Insight of these mechanisms could provide additional information on the optimal prescription of these novel modalities in training such as: rehabilitation of injuries, pre-conditioning and general training, whilst considering wider implications such as safety.

CHAPTER 2: Literature Review

2.1 Resistance Exercise

RE is the most potent stimulus to augment skeletal muscle growth and strength. Traditionally skeletal muscle size and strength increase when exercising at a load exceeding 70 % of a person's 1RM (McDonagh and Davies, 1984). However, many variables can be manipulated to cause RE induced muscle growth, with Kraemer (1983) outlining five fundamental principles: exercise mode, load (amount of resistance), volume (repetitions/sets x load), rest periods and exercise order.

The mechanical disruption of skeletal muscle fibres is critical to induce skeletal muscle hypertrophy (Goldberg et al., 1975), causing a cascade of anabolic and catabolic signalling pathways leading to a prioritisation of MPS over degradation (Schoenfeld, 2013), whereby only when MPS exceeds degradation can hypertrophy occur (Chesley et al., 1992). To reach MPS an intricate cascade of sequential factors take place, these are: muscle fibre activation; signalling caused by mechanical muscle fibre deformation, hormonal, metabolic and immune responses; resulting in transcription and translation (Spiering et al., 2008a) (Figure 2.1). The major signalling pathway regulating MPS is the mammalian target of rapamycin (mTOR) (McCarthy and Esser, 2010) however multiple other pathways are involved (mitogen-activated protein kinase [MAPK] and calcium [Ca^{2+}] dependent pathways) all acting in a synergistic manner (Schoenfeld, 2013).

Skeletal muscle hypertrophy compromises of a large range of molecular and cellular interactions; these will be discussed in brief following the RE biological paradigm (Figure 2.1), with a greater depth being provided in areas where the contribution of metabolic and oxidative stress can be advantageous.

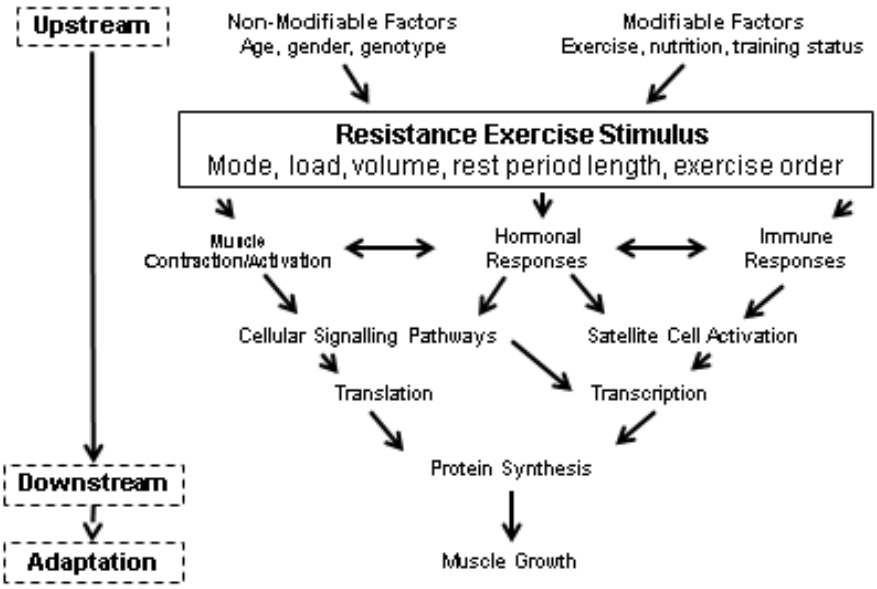

Figure 2.1. Biological paradigm of resistance exercise, with upstream stimuli (non-/modifiable factors and programme variables) modifying downstream biological stimuli. *Modified(Spiering et al., 2008a).*

2.1.1 Muscle Activation

Force production is the result of α-motor neurones activating muscle fibres with both neural firing frequency and motor unit size regulating the force produced. Under low force small motor units (fatigue resistant type I fibres) are recruited and as force increases a shift towards larger motor units (type II fibres) occurs (Henneman et al., 1965). It has been demonstrated that type II fibres have a greater hypertrophy potential (Staron et al., 1990, McCall et al., 1996), with Parkington et al. (2003) observing the up regulation of key signalling pathways (mTOR) to a greater extent in type II fibres despite all motor units being recruited. Therefore the activation of higher motor units through lifting heavy loads may be essential for optimal hypertrophy and strength gains, however it should be noted different muscles compromise of varied fibre type phenotypes (Thorstensson and Karlsson, 1976).

2.1.2 Hormonal Response

RE results in transient increases in endogenous anabolic hormones such as growth hormone (GH) (Hymer et al., 2001) , insulin-like growth factor-1 (IGF-1) (Kraemer et al., 1990) and testosterone (Tremblay et al., 2004), with magnitude being highly dependent upon Kraemer's five fundamental training principles (Kraemer and Ratamess, 2005). Although anabolic hormone increases occur alongside RE and thus hypertrophy recent evidence suggest they are not key mediators, West et al. (2009) demonstrate downstream anabolic signalling pathways involved in MPS can occur without the transient anabolic hormones.

2.1.3 Cellular Signalling Pathways

The mechanical signal caused by RE is converted by the affected cell into a biochemical event (mechano-transduction), enabling upstream independent signalling of pathways which can increase MPS (Fernandes et al., 2012). Specifically, mechano-transduction leads to an activation of the protein kinase-B mammalian target of rapamycin (Akt-mTOR) pathway, Akt precedes and upon activation phosphorylates mTOR. Once activated, mTOR increases protein metabolism and cell growth through phosphorylating the downstream targets 70kDa ribosomal protein S6 kinase (p70 S6K) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), which cause an increase in translational factors which increase MPS (Fernandes et al., 2012) (Figure 2.2). Direct activation of the ribosomal units (protein builders) via p70 S6K phosphorylation after mechanical loading was demonstrated by Baar and Esser (1999) which consequently correlated with increases in muscle mass, with further evidence has been provided through p70 S6K knockout (Aguilar et al., 2007). Not all mechanical stimuli can activate the Akt-mTOR pathway, typically only high load resistance exercise can (Dreyer et al., 2006, Eliasson et al., 2006), with type II fibres predominately exhibiting p70 S6K phosphorylation (Parkington et al., 2003).

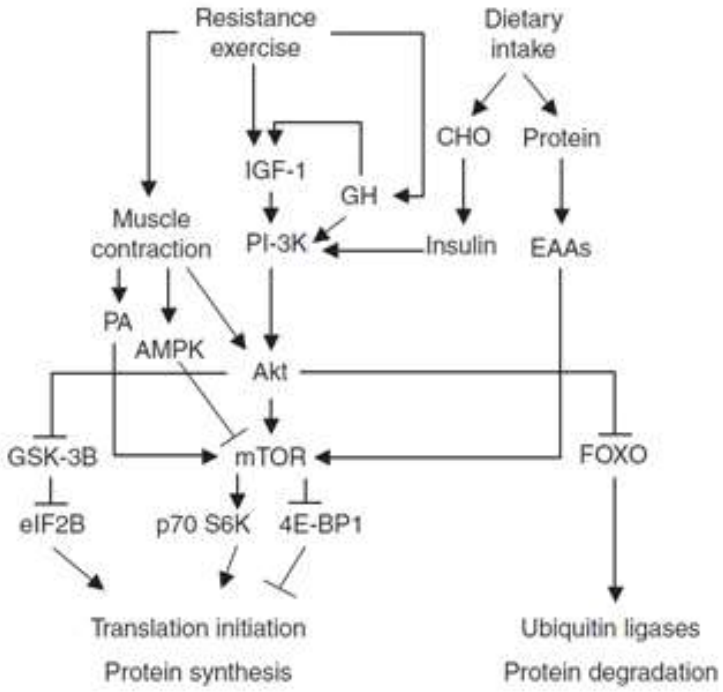

Figure 2.2. Model of how resistance exercise stimulates muscle hypertrophy through hormonal responses, mechanotransduction and dietary intake. Eukaryotic initiation factor 4E binding protein-1(4E-BP1), protein kinase B (Akt), carbohydrate (CHO), essential amino acids (EEAs), eukaryotic initiation factor 2B (eIF2B), fork-head box O transcription factor (FOXO), glycogen synthase kinase-3B (GSK-3B), mammalian target of rapamycin (mTOR), phosphatidic acid (PA), phosphatidylinositol-3 kinase (PI-3K), 70 kDa ribosomal protein S6 kinase (p70 S6K) (Spiering et al., 2008a).

2.2 Resistance Exercise with Blood Flow Restriction

BFR exercise is the application of an external pressure (tourniquet, inflatable cuff, elastic strapping) proximal to the exercising muscle, where the pressure applied is sufficient to maintain arterial inflow but prevent venous outflow, thus inducing a localised hypoxic environment (Pope et al., 2013). An influx of interest has manifested due to BFR training demonstrating significant increases in muscle hypertrophy and strength at training loads as low as 20% 1RM (Takarada et al., 2002, Takarada et al., 2004, Abe et al., 2005, Sumide et al., 2009). These recent findings contrast the ACSM (2009) position stand which recommends RE intensity should exceed 70% 1RM to induce optimal hypertrophy. With this in mind BFR represents an alternative option for those who cannot tolerate the traditional large mechanical loads required for muscle hypertrophy, for example the elderly and those rehabilitating injuries.

The application of BFR has demonstrated increases in muscular hypertrophy, strength and endurance in various exercise modalities such as walking (Abe et al., 2009), cycling (Abe et al., 2010) and circuit training (Ishii et al., 2005), however for the purpose of this review from this point forward only RE will be discussed.

2.2.1 Skeletal Muscle Hypertrophy

Many research groups have observed an increase in muscle cross sectional area (mCSA) as a result of BFR training with low loads. Abe et al. (2005) found two weeks of low intensity (20%) leg curls and squats with BFR increased mid-thigh muscles mCSA by 8.5 % compared to no change in a control group. Similar responses have been observed more recently, with mCSA of the extensors increases 6.6% in female netball players undergoing a low intensity (20% 1RM) BFR routine (Manimmanakorn et al., 2013a). More interestingly Takarada et al. (2000b) observed low intensity RE with BFR (30-50% 1RM) to increase mCSA of the upper arm to a similar magnitude as high intensity RE without BFR over a period of 16 weeks, however more recently these findings have not been replicated (Kim et al., 2012). Increases in strength are highly dependent upon increases in mCSA and neural adaptations (Schantz et al., 1983), therefore multiple authors have reported increases in muscular strength

following low intensity BFR training (Shinohara et al., 1998, Takarada et al., 2000b). The reader is directed to the review by Loenneke et al. (2012) for skeletal muscle hypertrophy and strength adaptations to BFR training.

2.2.2 Muscle Activation

With the addition of BFR to low intensity RE a hypoxic intramuscular environment ensues with the onset of multiple metabolic responses: increased adenosine triphosphate (ATP) hydrolysis and phosphocreatine (PCr) (Suga et al., 2012), decreased pH due to hydrogen ions ($H^{+)}$ (Suga et al., 2009) and increased BLa^{-} production (Fujita et al., 2007). This increase in metabolic stress lead Loenneke et al. (2011a) to suggest that the enhanced muscle adaptations from BFR training are the result of a metabolite/volume threshold, whereby the intramuscular environment causes an increase in type II fibre recruitment. Although intramuscular fatigue can occur during low intensity RE, during BFR this response is augmented and therefore a lower volume of exercise is required. Although increased muscle activation is likely to be involved in BFR muscular hypertrophy, when exercising to failure, low intensity BFR does not activate muscle to the same magnitude as high intensity RE (Cook et al., 2013).

2.2.3 Tissue Oxygenation

Despite the primary hypothesis for BFR adaptations being the accumulation of metabolites, few studies have investigated the skeletal muscle oxygenation. The observation of increased HHb in the muscle exposed to BFR would directly inform us that venous pooling is occurring. Originally Takano et al. (2005) reported that low intensity (30% 1RM) BFR caused a 22% decrease in mean maximal muscle oxygenation of the vastus lateralis during leg extensions. Exercise intensities of 50% (isotonic), 50% (isometric) and 80% 1RM (isotonic) without BFR showed decreases to a lesser extent at 24%, 28% and 36%, this decrease could be explained by increased intramuscular pressure which can restrict blood flow during RE.

More recently, Karabulut et al. (2014) investigated initial restriction pressures on tissue oxygenation, participants performed 4 sets (30,15,15,15 reps, 1 min rest) of low load (20% 1RM) isotonic knee extensions. A higher initial starting pressure

(65mmHg) elicited greater increases in HHb and THb compared to a lower starting pressure (45mmHg), however there was no difference between conditions for O_2Hb. During rest periods a trend occurred for increased HHb and THb which was subsequently decreased during muscular contractions, presumably via a skeletal muscle pump action. In another methodological investigation, Cayot et al. (2014) suggests there could be an intensity threshold for the accumulation of HHb during BFR. When HHb (as a % of maximal plateau) was measured during 5 second isometric knee extensions at both 60% and 80% MVC there was no difference between control and when BFR (130% of systolic blood pressure) is applied immediately before exercise. When BFR was applied 5 minutes before exercise a greater metabolic strain (HHb and THb) was observed at all intensities (20, 40, 60, 80 % MVC)

2.2.4 Hormonal Response

Multiple studies have displayed that low intensity exercise with BFR promotes a favourable anabolic endocrine response (Takarada et al., 2000a, Abe et al., 2005, Pierce et al., 2006). An early seminal study by Takarada et al. (2000a) reported plasma GH levels to rise 290 time greater that baseline after completing 5 exhaustive sets of leg extensions (20% 1RM), with no change in the control group. A low intensity (30% 1RM) BFR exercise regime induced a 4-fold increase in GH post exercise, compared to high intensity RE (70% 1RM) which saw no change. These post exercise elevations are potentially mediated via the accumulation of H^+ and lactate in the blood, where adecrease in pH can mediate a GH response through a chemo-reflex stimulation which is caused through intramuscular metaboreceptors and group III/IV afferents (Viru et al., 1998, Schoenfeld, 2013). Through enhanced GH response the synthesis of IGF-1 occurs which plays a role in downstream mechanisms for increased MPS, low intensity BFR training has shown to increase IGF-1 (Takano et al., 2005, Abe et al., 2005) in a similar manner to high intensity RE (Borst et al., 2001).

2.2.5 Cellular Signalling Pathways

Activation of key intracellular signalling pathways to increase MPS typically occur during high load RE (Dreyer et al., 2006), a contradiction to this is low load BFR

(Fujita et al., 2007, Fry et al., 2010, Wernbom et al., 2013). For the first time (Fujita et al., 2007) demonstrated that low load BFR (30% 1RM) increased the phosphorylation of p70 S6K the key downstream regulator of mTOR; this subsequently increased MPS 3 hours post exercise, there were no changes in the control group. Similar responses were presented by Fry et al. (2010) and Wernbom et al. (2013) both concluding that the increased intracellular signalling is likely to be responsible for the enhanced hypertrophic effect of BFR. To further support the previous statements, Nielsen et al. (2012) observed an increase in satellite cells , myonuclei addition and myofibre hypertrophy in human skeletal muscle following low load (20% 1RM) BFR, with no change in control group however there was no high load RE group for comparison.

2.2.6 Perceptual Response

The perceptual response of RE can be useful when monitoring exercise intensity, its physiological demands and may inform which exercise modality you prescribe to certain populations (Scott et al., 2014). When prescribing BFR many variables could affect the rate of perceived exertion (RPE) or perceived pain (PP), these include cuff width (Loenneke et al., 2013), restriction pressure (Yasuda et al., 2010a) and exercise load (Loenneke et al., 2015). Multiple studies have reported similar levels of RPE (Wernbom et al., 2006, Sumide et al., 2009, Wernbom et al., 2013) and PP (Manimmanakorn et al., 2013a) during BFR when compared to an intensity matched control. One study utilising knee wraps rather than an inflatable cuff expressed higher levels of RPE and PP (Loenneke et al., 2011b) with BFR compared to without, this could be caused by an inability to quantify restriction pressure. Monitoring RPE and PP throughout the use BFR could help improved exercise prescription and inform future methodology.

2.3 Resistance Exercise with Systemic Hypoxia

The beneficial outcomes as a result of inducing a localised hypoxic environment (BFR) during RE training are plentiful (section 2.4); in the advent of this it is plausible to assume similar benefits could arise from RE in systemic hypoxia. Intermittent hypoxic training (decrease in O_2 during training) has shown to increase aerobic (Meeuwsen et al., 2001) and anaerobic (Hendriksen and Meeuwsen, 2003) performance, largely through enhanced metabolic capacity and oxygen delivery (Hoppeler et al., 2008). However, little research is available with respect to intermittent hypoxic resistance exercise (IHRE), using hypoxic devices to provide a systemic normobaric (sea level) hypoxic environment either through nitrogen dilution or oxygen extraction (Scott et al., 2014).

2.3.1 Skeletal Muscle Hypertrophy

The first study to investigate IHRE (Friedmann et al., 2003) saw no changes in mCSA following a low intensity (30% 1RM) 4 week knee extension programme, where IHRE was performed at a F_IO_2 = 12 %. Following a higher intensity programme (squats, 3 sets of 10RM, 3 x week, 6 weeks) at an F_IO_2 = 15 %, Ho et al. (2014b) reported no changes in functional strength or body composition. However, no significant changes were present in the normobaric control group either. Contrary to the above Manimmanakorn et al. (2013a) exposed female netball athletes to low intensity (20% 1RM) leg extension exercises for 5 weeks , with mCSA increasing significantly with IHRE (6.1 ± 5.1%) and BFR (6.6 ± 4.5%) compared to normobaric control (2.9 ± 2.7%), increases in muscular strength/endurance were also present for IHRE and BFR. These findings were subsequently replicated by the same research group (Manimmanakorn et al., 2013b). Two further studies have reported positive morphological adaptations following IHRE, Nishimura et al. (2010) reported increased mCSA of the elbow flexors/extensors and 1RM in response to a 6 week moderate intensity (70% 1RM) training regime at a F_IO_2 = 16 %, a matched normobaric control saw increases to a lower proportion. After an 8 week elbow extension programme (10RM, 3 sets, 3 x week, F_IO_2 = 12.9%), Kurobe et al. (2014) reported that bicep and tricep thickness increased to a greater extent in IHRE compared to normobaric control, as did 10RM.

The variation between investigators could be accounted for by exercise programme duration, the programme Friedmann et al. (2003) implemented was 4 weeks where as Nishimura et al. (2010),Manimmanakorn et al. (2013a) and Kurobe et al. (2014) were 5, 6 and 8 weeks respectively. In addition the interest recovery period length of 2 minutes used by Ho et al. (2014b) is likely to be sufficient enough to remove metabolites which are proposed to augment the beneficial metabolic and hormonal response of IHRE.

2.3.2 Muscle Activation

Only one study has investigated muscle activation in response to IHRE training, following 5 weeks of low intensity RE (20% 1RM) with IHRE, BFR or normobaric control, electromyographic (EMG) activity significantly increased in all conditions, with the greatest increase present in the BFR condition, however IHRE increased compared to control (Manimmanakorn et al., 2013b). Although not during RE, preferential recruitment of type II fibres has been observed during cycle ergometry in hypoxia (F_IO_2 = 13.5 %) (Melissa et al., 1997).

2.3.3 Tissue Oxygenation

Ivamoto et al. (2014) reported that a decrease in arterial oxygen saturation (S_pO_2) caused by hypoxic conditions (F_IO_2 = 13 %) had no effect on isokinetic strength or fatigue of the knee extensors, muscle oxygen delivery may have been maintained enough to prevent fatigue. As far as the author knows, there have been no investigations into skeletal muscle oxygenation during IHRE, further research is required to elucidate the possible mechanisms that skeletal muscle oxygenation contribute to IHRE hypertrophy.

2.3.4 Hormonal Response

Similar hormonal responses to IHRE at both low (Kon et al., 2012) and high intensities (Kon et al., 2010) have been observed to those with BFR (section 2.4), promoting an anabolic endocrine environment for skeletal muscle adaptation. Initially, Kon et al. (2010) reported IHRE (F_IO_2 = 13 %) at 70% 1RM augmented GH response 15 and 30 minutes post exercise whereas normobaric control did not, this

could have been facilitated by enhanced blood lactate (BLa^-) accumulation (Elias et al., 1997). The same group went on to investigate IHRE at a low intensity yielding similar findings, this time with increased GH immediately post and 15 minutes post exercise compared to control, however on this occasion although there was an increase there was no difference in BLa^-. In contrast Ho et al. (2014a) concluded low intensity (30% 1RM) IHRE does not induce a greater anabolic response post exercise compared to normobaric control, both groups experienced similar increases in GH and testosterone. Disparities between the Kon lead research group and Ho et al. (2014a) could be due the later having 90 seconds rest between sets rather than 60 seconds. In the Ho et al. (2014a) study both IHRE and control experienced similar BLa^- responses; the prolonged rest period could have caused increased metabolite clearance the proposed mechanism for augmented endocrine response.

2.3.5 Cellular Signalling Pathways

To date only one study has investigated the intracellular signalling response to IHRE. Etheridge et al. (2011) implemented a moderate intensity unilateral leg RE regime (70% 1RM, 6 sets, 8 reps), participants were exposed to a F_IO_2 = 12 % for 3.5 hours. IHRE caused an increase in downstream mTOR p70 S6K phosphorylation however blunted MPS in a SpO_2 dependent manner, the author speculated there could be an unknown signalling interaction cause by exposure to IHRE as there was no change in MPS when exposed to hypoxia alone. Hypoxic-inducible factor 1 (HIF-1) is a transcriptional regulator of oxygen homeostasis, upon chronic or intermittent exposure to hypoxia HIF-1 can mediate the maladaptive response, therefore the prolonged 3.5 hour exposure to hypoxia could be the causes of the blunted anabolic response (Semenza, 2009).

2.5.6 Perceptual Response

Although IHRE presents additional metabolic stress both Nishimura et al. (2010) and Kon et al. (2012) reported that there was no significant difference in RPE compared to normobaric control. In contrast Manimmanakorn et al. (2013a) have observed significant increased PP during IHRE compared to both BFR and control, this observation is perplexing as one would assume an increased PP in the BFR

condition as well. Future research should monitor perceptual response to IHRE to inform future prescription.

2.4 Oxidative Stress

Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the physiological capacity of the system to eliminate or neutralise these molecules. ROS such as singlet oxygen (·O), superoxide radicals ($O_2^{\cdot -}$), hydroxyl radicals (·OH) and hydrogen peroxide (H_2O_2) occur through natural oxidative mitochondrial metabolism and are disposed of through the bodies endogenous antioxidant defence system as well as exogenous dietary intake (Bloomer and Goldfarb, 2004). Exposure to oxidative stress through muscle disuse and ischemia can result in macromolecular damage (nucleic acids, lipids, proteins) and is implicated in various disease states including atherosclerosis, diabetes, neurodegeneration, cancer and ageing (Ray et al., 2012). In a paradoxical manner, ROS production also plays a role in the regulation of key signalling pathways involved in skeletal muscle adaptation through contraction (Powers et al., 2010).

During resting conditions the production of ROS are relatively low allowing the bodies endogenous antioxidant system to neutralise these. However, upon the onset of exercise the bodies' uptake of oxygen dramatically increases. Whole body oxygen consumption can increase by 10-15 folds during aerobic activity, resulting in a 100 fold increase in oxygen flux to the active muscle groups (Sen, 1995). When ROS production outweighs the antioxidant buffering capacity of the muscle, oxidative stress occurs. It should be noted that the production of ROS also occurs through the autoxidation of haemoglobin, catecholamine's and thiols (Cohen and Heikkila, 1974).

2.4.1 Antioxidant defences

Many antioxidant defence systems exist within the body to prevent oxidative stress, endogenously these include glutathione reductase (GR), catalase and superoxide dismutase, with exogenous sources available from vitamin E, vitamin C and β-

carotene (Chance et al., 1979). Due to its use within the oxidative stress literature and its use in the study the glutathione system will be described in detail below.

Glutathione (GSH) is believed to be essential in maintaining cellular homeostasis during oxidative stress, as the most abundant non-protein thiol in the cell, synthesis takes place within the cytosol and distributed in a intracellular manner (Sastre et al., 1996). In human tissue GSH is predominantly found in its reduced form (99%) however this changes when it is degraded to its oxidised form (GSSG), taking place in extracellular spaces allowing for membrane transformation (Owen and Butterfield, 2010). GSH can neutralise hydroxyl radicals through enzymatic glutathione peroxidase (GPx) or non-enzmatic reactions generating GSSG, which is then available to be recycled back into GSH (Elokda and Nielsen, 2007).

2.4.2 Oxidative Stress and Muscle Hypertrophy

The production of $O_2^{\cdot -}$ radicals and other ROS takes place in multiple places within the skeletal muscle fibre including the mitochondria, cytosol, sarcoplasmic reticulum and sarcolemma (Powers et al., 2010). Interestingly the mitochondria within type II fibres generate greater levels of $O_2^{\cdot -}$ radicals and ROS compared to type I fibres (Anderson and Neufer, 2006), through electron leakage. There is much debate as to the role of ROS and muscular adaptation. It is thought that ROS play a major role in muscle hypertrophy through influencing many transcription factors altering hypertrophy related genes (Torres and Forman, 2003). Handayaningsih et al. (2011) treated C2C12 myocytes with H_2O_2 enhanced IGF-1 phosphorylation of its receptor, whereas treatment with antioxidants provided the opposite response and down regulated the Akt-mTOR pathway and phosphorylation of p70 S6K. Interleukin-6 (IL-6) is an essential regulator of muscle stem cells (satellite cells), ROS stimulate the release of IL-6 from muscle myotubes (Kosmidou et al., 2002) and when IL-6 is knocked out a blunted hypertrophy response occurs.

The argument for ROS role in hypertrophy is strengthened through the investigation of exogenous antioxidant supplementation, Gomez-Cabrera et al. (2008) administered vitamin C causing a decrease in peroxisome proliferator-activated receptor-y co-activator-1α (PGC-1α), skeletal mitochondrial biogenesis and antioxidant enzymes. In a similar manner Ristow et al. (2009) concluded that

antioxidant supplementation cannot retard skeletal muscle adaptation and the regulation of endogenous antioxidant defences such as the glutathione system.

2.4.3 Resistance Exercise and Oxidative Stress

A multitude of studies have taken place investigating the oxidative stress response to various resistance exercise routines (Table 2.1), these will be discussed below grouped by type of muscular contraction. The oxidative stress biomarkers discussed below are in blood unless otherwise stated.

2.4.4 Isometric Exercise

During isometric exercise the joint angle and muscle length remains constant. The role of oxidative stress and isometric exercise was first investigated by Sahlin et al. (1992), who utilised intermittent (10 sec on, 10 sec off) knee extensions at 30 % MVC for 80 minutes. The group found no changes in oxidative stress markers malonadialdehyde equivalents (MDA), total glutathione (TGSH) or oxidised glutathione (GSSG). During aerobic exercise intensity is a dependent factor in oxidative stress accumulation (Leaf et al., 1997), therefore the low MVC chosen by Sahlin et al. (1992) could explain why they saw no change.

Alessio et al. (2000) increased the load (50% MVC) and had participants performing intermittent (45 sec on 45 sec off) isometric handgrips, exercising for approximately 15 minutes (matched to a VO_{2MAX} protocol). Immediately post exercise there was an increase in lipid peroxidation which was maintained for an hour, there was a small but non-significant increase in protein oxidation. The author hypothesised that the increase in ROS was due to the ischemic reperfusion prolonged isometric contractions cause and the built up of metabolites which could affect the pro-oxidant/antioxidant balance (P/A ratio) (Alessio et al., 2000). Both Dousset et al. (2002) and Steinberg et al. (2002) examined the effect of isometric handgrip exercise as well, with both observing oxidative stress in the form of lipid peroxidation products (TBARS) despite differences in exercise intensity (60% MVC and 100% MVC respectively).

More recently Zembron-Lacny et al. (2008) investigated isometric quadriceps contractions at 100% MVC (10 sec, 3 sets) at different angles (30° and 75°). In line

with previous research (Dousset et al., 2002, Steinberg et al., 2002) the group found increases in TBARS immediately post exercise along with increases in other oxidative stress markers.

2.4.5 Eccentric Exercise

During eccentric contractions the muscle elongates whilst remaining under tension with the opposing force being greater than the force the muscle produces. Due to the fact that eccentric contractions under high loads typically induce muscle damage, the magnitude of ROS production could be increased as a result of inflammatory pathways and disruptions in calcium homeostasis (Powers and Jackson, 2008).

The majority of the current research has observed the oxidative stress response to eccentric contractions of the elbow flexors with mixed results. Childs et al. (2001) saw no changes in total antioxidant capacity (TAC) and glutathione peroxidase (GPx) following 70 reps (100% MVC), however these were not measured immediately post exercise, rather alongside the monitoring of muscle damage response. Two days – five days post exercise there was still an increase in lipid hypoperoxides (LH) correlating in a linear fashion with creatine kinase (CK) suggesting an ROS involvement in muscle damage (Childs et al., 2001). In another muscle damage protocol (Lee et al., 2002) (60 reps, 150% maximum isometric force [MIF]), there was little to no oxidative stress response with no changes in glutathione status (GSSG:TGSH). This could be due to the blood withdrawal taking place 20 minutes post exercise allowing time for GSSG to be converted back to TGSH. Lee et al. (2002) saw an increase in protein carbonyls (PC) 1-2 days post exercise and not immediately post, again this suggests that there could be an inflammatory oxidative stress response following muscle damage. Finally Lee and Clarkson (2003) enforced a comparable regime to the above, an increase in TGSH was present immediately post – 4 days but only in participants who had chronically low TGSH. In contrast to the Lenn et al. (2002) saw no changes in MDA or TBARS following a muscle damage protocol.

Still implementing eccentric exercise however using the knee extensors, following an intensive protocol (70 reps, 100% MVC) Child et al. (1999) did not see a change in MDA or TAC in blood or muscle. From the above literature it is clear that the

oxidative stress response immediately following eccentric exercise is minimal however does occur through the duration of muscle damage recovery, possibly through inflammatory pathways.

2.4.6 Whole Body Resistance Exercise

The research discussed in this section will investigate isotonic (concentric/eccentric) resistance exercise regimes, those of which are the most prescribed as a component of fitness programmes.

Exercise regimes incorporating multiple resistance exercises have yielded mixed results. An increase in MDA was present immediately after and up to two days following a hypertrophy circuit routine (McBride et al., 1998), whereas a similar routine saw no increases in F2-isoprostanes or ferric reducing ability of plasma (FRAP) (McAnulty et al., 2005). F2-isoprostanes are a sensitive marker of lipid peroxidation (oxidative stress) which are seen to be more reliable than TBARS and MDA which experience high assay variability. Zembron-Lacny et al. (2008) unlike the previous two studies discussed only used four exercises rather than eight, immediately post exercise increases in TBARS, P/A ratio, catalase (CAT) increased with decreases in glutathione peroxidase (GPx) and superoxide dismutase (SOD). It is likely that the differences in protocols explain the varied results.

Four different studies have explored the oxidative stress response to similar hypertrophy squat protocols (Bloomer et al., 2005, Bloomer et al., 2006, Bloomer et al., 2007, Hudson et al., 2008), all of these studies with the exception of Bloomer et al. (2006) saw transient increases in PC. Interestingly Hudson et al. (2008) exhibited that the magnitude of PC response was intensity dependent, in a work matched manner a strength squat protocol (90% 1RM, 3 reps, 11 sets, 5 minutes rest) displayed higher post exercise PC concentrations that a hypertrophy protocol (75% 1RM, 4 sets, 10 sets, 90 seconds rest). Bloomer et al. (2005) saw an increase in GSSG:TGSH immediately post exercise which was the only oxidative stress marker to exhibit a response in the above studies discussed above.

From the review of the above exercise modalities it is clear that there is an oxidative stress response to resistance exercise although equivocal. It is clear that the response is intensity dependent and not entirely reliant on aerobic metabolism

(Quindry et al., 2003). An additional factor for consideration is the biomarkers which are selected to interpret oxidative stress.

Table 2.1. Effect of resistance exercise on oxidative stress response in humans.

Study	Participants	Resistance Exercise	Intensity	Duration	Blood Samples	Oxidative Stress Biomarkers
Sahlin *et al* . (1992)	(n = 7)	Isometric knee extensions	30 % MVC	10 sec on - 10 sec off 80 mins	Pre, 20,40, 60, 80 min DE	MDA ↔ TGSH ↑(80 min DE) GSSG ↔
McBridge *et al.* (1998)	Trained (n = 12)	Full body (8 exercises)	10 RM	3 sets 10 reps 1 min rest (circuit)	Pre, IP, 6hr, 1d,2d	MDA ↑(IP, 6hr, 1d)
Child *et al.* (1999)	Untrained (n = 8)	Eccentric contractions knee extendors	100 % MVC	70 reps 10 sec rest	Pre IP	MDA ↔ TAC ↔
Alessio et al. (2000)	(n = 12)	Isometric handgrip exercise	50 % MVC	45 sec contraction 45 sec rest (time equal to VO2max protocol)	Pre IP 1hr	MDA ↔ (IP, 1hr) LH ↑(IP, 1hr) PC ↔ (IP, 1hr) ORAC ↔ (IP, 1hr)
Childs *et al.* (2001)	Untrained (n = 14)	Eccentric contractions elbow flexors	80 % MEF	3 sets 10 reps 2 min rest	Pre, 2d,3d, 4d,7d	TAC ↔ GPx ↔ LH ↑ (2d,3d,4d)
Steinberg *et al.* (2002)	(n = 7)	Isometric handgrip exercise	100% MVC	3 mins 1 sec on 1 sec off	Pre IP	TBARS ↑ GSH ↓
Lenn *et al* . (2002)	Untrained (n = 22)	Eccentric contractions elbow flexors	100 % MEF	50 reps 90°s-1	Pre, IP, 3hr, 1d, 2d, 3d	MDA ↔ TBARS ↔
Dousset *et al.* (2002)	(n = 8)	Isometric handgrip exercise	60% MVC	Until failure (42±5 sec)	Pre IP	TBARS ↑
Lee *et al* . (2002)	Trained (n = 8)	Eccentric contractions elbow flexors	150% MIF	60 reps (90° - 170°) 3 sec contractions 7 sec rest	Pre, IP, 1d, 2d, 3d, 4d	GSSG:TGSH ↔ PC ↑ (1d, 2d)
Lee and Clarkson (2003)	Untrained (n = 60)	Eccentric contractions elbow flexors	100% MIF	50 reps 3 sec contraction 12 sec rest	Pre, IP, 1d, 2d, 3d, 4d	TGSH ↑(All time points)

Bloomer *et al.* (2005)	Trained (n = 10)	Squats	70% 1RM	Until failure 90/120 sec rest 30 mins	Pre, IP,1hr, 6hr, 1d	PC ↑(6hr, 1d) GSSG:TGSH ↑(IP) MDA ↔ OHdG ↔ GSH ↓ (IP)
McAnulty *et al* . (2005)	(n = 15)	Full body (8 exercises)	40% 1RM (set 1) 60% 1RM	4 sets 10 reps (circuit)	Pre IP	F2-isoprostanes ↔ FRAP ↔
Bloomer *et al.* (2006)	Trained (n = 12)	Squats	70% 1RM	Until failure 6 sets 3 mins rest	Pre, IP, 30min, 1d, 2d	PC ↔ MDA ↔
Bloomer *et al* . (2007)	Trained (n = 13)	Squats	70 % 1RM + body mass	15 reps 5 sec rest	Pre IP	PC ↑ MDA ↔ 8-OhdG ↔
Hudson *et al.* (2008)	(n = 10)	Hypertrophy Squat Protocol	75% 1RM	4 sets 10 reps 90 sec rest	Pre IP 1hr	PC ↑ LH ↔
Zembron-Lacny *et al.* (2008)	Counterbalanced (n = 14)	Multi-joint (shoulder press, deadlift, bench press)	50% - 92% 1RM	5 - 7 sets Until failure	Pre IP	TBARS ↑ SOD ↓(↑single joint) CAT ↑ GPx ↓ P/A ratio ↑(↔ single joint)
		Single-joint isometric quadricep contractions	100% MVC	3 sets 10 secs 30° & 75 °		
Zembron-Lacny *et al.* (2009)	Trained (n = 13)	Single-joint isometric quadricep contractions	100% MVC	3 sets 10 secs 30° & 75 °	Pre IP 1d	TT ↔ GSH ↔ (1d ↓) GR ↔ (1d ↑) GPx ↓ TBARS ↔ PC ↔
		Single-joint isokinetic sequence	100% MVC	60, 120, 180, 210, 450 °.s^{-1}		

Maximal voluntary contraction (MVC), maximal isometric force (MIF), one repetition max (1RM), during exercise (DE), malondialdehyde equivalents (MDA), lipid hydroperoxides (LH), protein carbonyls, oxygen radical absorbance capacity for peroxyl radicals (ORAC), lipid peroxidation products (TBARS), oxidised glutathione (GSSG), glutathione peroxidase (GPx), reduced glutathione (GSH), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), plasma total thiols (TT), ferric reducing ability of plasma (FRAP), 8-hydroxydeoxyguanosine (8-OhdG), total antioxidant capacity (TAC), pro-antioxidant ration (P/A ratio), increase post exercise(↑), decrease post exercise (↓), no change post exercise (↔).

2.4.7 Blood Flow Restriction and Oxidative Stress

There is limited research into the response of BFR on oxidative stress (Table 2) therefore further investigation is required. Initially Takarada et al. (2000a) saw no changes in lipid peroxide following 5 sets of bilateral leg extensions to exhaustion under low load (20% 1RM) with or without BFR. One critique of this study could be that there was no moderate/high load group to make comparison. Goldfarb et al. (2008) did utilise a moderate load group (70% 1RM) with a protocol consisting of 3 sets of bicep curls and calf extensions to failure. The moderate load group had increases in PC and GSSG:TGSH immediately post and 15 minutes post exercise whereas the low load (30% 1RM) with BFR saw no changes. Interestingly the addition of a control group with no exercise and only occlusion resulted in similar results as the high load condition. The authors postulated that in the low load BFR condition the muscular contractions were able to overcome the resistance to venous outflow thus diminishing the oxidative stress response (Goldfarb et al., 2008).

More recently (post completion of this study) Garten et al. (2015) also found that BFR in the absence of exercise increases PC, however unlike Goldfarb et al. (2008) not GSSG:TGSH. Moderate load RE (70% 1RM) with BFR caused the highest oxidative stress response with increases in PC and GSSH:TGSH significantly higher than low load (30% 1RM) with BFR. In a similar manner to Goldfarb et al. (2008) the addition of BFR to low load RE caused a blunted response in PC, with exercise intensity acting as the dependent factor in the magnitude of oxidative stress response. To conclude the addition of BFR to resistance exercise, especially at low intensities can blunt the ROS response; this could affect the amplitude of subsequent intracellular signalling and adaptation.

Table 2.2. Effect of blood flow restriction resistance exercise on oxidative stress response in humans.

Study	Participants	Conditions	Resistance Exercise	Intensity	Duration	Occlusion Method	Blood Samples	Oxidative Stress Biomarkers
Takarada *et al* *(* 2000)	Trained (n = 6)	LR LR-BFR	Bilateral leg extensions (CON = no. reps in BFR)	20% 1RM	Until failure 5 sets 30 sec rest	Wide 33mm 214 ± 7.7 mmHg Upper thigh both legs Rest Exposure	Pre, IP, 15, 30, 45, 60, 90, 120min, 1d	BFR - LP ↔ (over time) CON - LP ↔ (over time)
Goldfarb *et al.* (2008)	Trained (n = 7)	CON-BFR	No exercise	n/a	n/a	Same time as LR-BFR	Pre IP 15min	*CON-BFR:* PC ↑(IP, 15) GSSG:TGSH ↑ (IP, 15)
		LR-BFR	Bicep curls Calf extensions	30% 1RM	Until failure 3 sets 1min rest	Width n/a Arm = SYS - 20 mmHg Leg = SYS + 20 mmHg Rest exposure		LR-BFR: PC ↔ GSSG:TGSH ↔
		MR		70% 1RM				MR-BFR: PC ↑(IP, 15min) GSSG:TGSH (IP, 15min)
Garten *et al.* 2015	(n = 12)	CON-BFR CON	No exercise	n/a	n/a			CON-BFR: PC ↑ GSSG:TGSH↓
		LR-BFR LR	Bicep curls	30% 1RM	Until failure 3 sets 1 min rest	Width n/a Arm = SYS - 20mmHg Rest Exposure	Pre IP	LR-BFR: PC↓ compared to LR GSSG:TGSH ↔
		MR-BFR MR		70% 1RM				MR-BFR: PC ↑compared to without bfr GSSG:TGSH ↑compared to LR-BFR but not

Light resistance exercise (LR), moderate resistance exercise (MR), blood flow restriction (BFR), no exercise (CON), one repetition max (1RM), systolic blood pressure (SYS), immediately post (IP), lipid peroxide (LP), protein carbonyls (CP), oxidised to total glutathione ratio (GSSG:TGSH).

2.4.8 Systemic Hypoxia and Oxidative Stress

Traditionally, an increased supply of oxygen to the tissue as a result of exercise is considered the primary reason for oxidative stress due to increased mitochondrial respiration (Sinha et al., 2009). Through different mechanisms, exposure to hypoxia can result in similar ROS production and subsequent oxidative stress (Bakonyi and Radak, 2004).

The oxidative stress response to passive, aerobic and anaerobic exercise hypoxic exposure is well documented (Joanny et al., 2001, Moller et al., 2001, Bakonyi and Radak, 2004). The prolonged passive exposure to hypoxia results in decreased SpO_2 and an increased formation of ROS, oxidative damage ensues due to compromised endogenous antioxidant defences (Bakonyi and Radak, 2004). The main source of ROS during exercise in hypoxia are believed to be derived through reperfusion, where the production of xanthine dehydrogenase take place, this is catalysed by oxidase to produce ROS ($O_2^{\cdot -}$ and H_2O_2) (Wozniak et al., 2001). Currently, no studies have investigated the oxidative stress response of addition of IHRE, however we currently know that the addition of hypoxia to can increase oxidative response in aerobic (Wozniak et al., 2001) and anaerobic (Joanny et al., 2001), thus we can hypothesis a similar result with IHRE.

2.5 Synthesis

Through reviewing the literature it is clear that there are areas where more insight can be provided with regards to resistance exercise with blood flow restriction exercise and in systemic hypoxia.

Specifically, the current study will observe the tissue oxygenation response to both exercise modalities defining whether there is change and whether alongside this change there is also an oxidative stress response.

2.6 Aims and Hypothesis

This thesis proposes to:

1) Investigate the proposed mechanism (venous pooling) of BFR through examining skeletal muscle oxygenation (TSI, O_2Hb, HHb, THB) before, during and after handgrip RE.

2) Examine whether the addition of BFR or HYP to handgrip RE would alter the oxidative stress response, when measured by GSSG:TGSH immediately after exercise.

3) Observe the perceived pain response during handgrip RE alone and with the addition of BFR or HYP.

It was therefore hypothesised that:

- The addition of BFR would increase THb/HHb and decrease TSI/O_2Hb compared to both CON and HYP before, during and after handgrip RE.

- The addition of HYP would not increase THb/HHb and decrease TSI/O_2Hb compared to CON before, during and after handgrip RE.

- Handgrip RE alone would elicit an oxidative stress response as measured by an increase in GSSG:TGSH, with the addition of BFR this response would be lower.

- Perceived pain would be greater during handgrip RE with the addition of BFR when compared to both HYP and CON. There would be no difference in perceived pain between CON and HYP.

CHAPTER 3: Methodology

3.1 Participants

Recreationally active healthy males (n = 8, 5-10 $h.wk^{-1}$ physical activity) were recruited to partake in the study via convenience sampling. Prior to participation the purpose, procedures and potential risks were communicated verbally to the participants and through a detailed information sheet (Appendix A) and subsequently written informed consent (Appendix B) was obtained in line with the Declaration of Helsinki. Participants could communicate any concerns verbally with the research team, and were able to withdraw at any time without explanation or disadvantage. Ethical approval was obtained from the University of Bedfordshire's Institute for Sport and Physical Activity Research (ISPAR) ethical committee (approval no. 2013SPA009). Medical screening prior to participation via a physical activity readiness questionnaire (PAR-Q) and blood analysis questionnaire (Appendix C and D) took place to ensure the safety of the researcher and participants. Those with high blood pressure (≥ 140/90 mmHg) were excluded from participation. All participants were non-smokers, free from musculoskeletal injury and any known medical conditions.

Prior to experimental participation pre-test instructions were provided and adherence tested (Appendix E). Participants were required to refrain from dietary/vitamin supplementation (anti-oxidants, nitrates, protein, branch chain amino acids) (Ji, 1999) , ergogenic aides (L-Arginine, β- Alanine, creatine) (Fayh et al., 2013) and exposure to extreme environments (hypoxia, hyperthermia, hypothermia) (Siervo et al., 2014). Additionally, participants were required to refrain from strenuous exercise, caffeine and alcohol 72 hours prior to experimental procedures. Apparent adherence to these pre-test procedures was 100% in all instances. Anonymity and confidentiality was preserved throughout, with all data and participant information collected stored on a password protected laptop only accessed by the primary researcher.

3.2 Anthropometric Data

During the initial visit body mass (kg) and height (cm) were measured pre-prandial utilising Digital Tanita scales (BWB0800, Allied Weighing, UK) and a wall mounted Stadiometer (Holtain Ltd, UK) respectively. Acquisition of blood pressure was obtained automatically following 10 minutes relaxed sitting, in line with manufacturer's instructions (M5-I, Omron, Cranlea, UK). Measures were taken in triplicate and an average taken (Table 3.1).

Table 3.1. Participant anthropometric data (n = 8).

Measure	Mean	SD	Range
Age (y)	22.6	0.9	21 - 24
Height (cm)	174.6	5.9	168 - 185
Mass (kg)	76	10.4	61.4 - 90.2
Diastolic BP (mmHg)	81.4	5.4	74 - 91
Systemic BP (mmHg)	125.1	5.7	117 - 134

3.3 Experimental Design

The study implemented a randomised design for the following conditions: control (CON), blood flow restriction (BFR) and hypoxia (HYP) (Figure 3.1A). Participants attended the Sport and Exercise Science Laboratories on five occasions (Familiarisation one, Familiarisation two, experimental trial one, experimental trial two, and experimental trial three), before 13:00 pm. During familiarisation one in addition to anthropometric data collection (section 3.2), participants also performed hand grip MVCs and the handgrip exercise protocol (section 3.4 & 3.5). The second familiarisation session was identical to familiarisation one except there were no anthropometric measures. Visits three, four and five compromised of the randomised experimental procedures (section 3.6, 3.7, 3.8), all of which took place at least 48 hours apart at the same time of day (before 13:00pm) to minimise circadian variation (Teo et al., 2011).

3.4 Assessment of Handgrip Strength

Handgrip strength was measured using a grip force transducer (AD Instruments, Australia) connected directly to a Power Lab 25T system (AD Instruments, Australia) where output could be visually seen on a monitor using Lab Chart 6 (AD Instruments, Australia). Following 15 low intensity contractions to warm up, participants were directed to perform a maximal voluntary contraction (MVC) in the supine position for 3 seconds using a randomised arm with 30 seconds rest, in triplicate. The average of the three trials was considered as the participants MVC. In familiarisation session one participants practiced their MVC, with the MVCs from familiarisation two taken forward to be used in the exercise protocol.

3.5 Experimental Procedures

All participants rested for 15 minutes in the supine position prior to and during the experimental procedure in a low light controlled laboratory environment. The experimental arm was chosen in a randomised fashion and used for all subsequent conditions. All conditions utilised the same exercise protocol (section 3.6) which was performed either with BFR (section 3.7), with HYP (section 3.8) or with exercise only (CON condition). Prior to the handgrip exercise the researcher took baseline NIRS measurements (as described in section 3.9) and obtained a baseline blood sample (see section 3.10). Exposure to the experimental condition (CON, BFR, HYP) began with measurement of haemoglobin variables alongside the 5 minute rest period after which the handgrip exercise protocol commenced. Upon cessation of the exercise protocol the condition exposure stopped (BFR, HYP) and a 10 minute recovery period pursued. The post exercise blood sample was collected 5 minutes into the recovery period (Figure 3.1B).

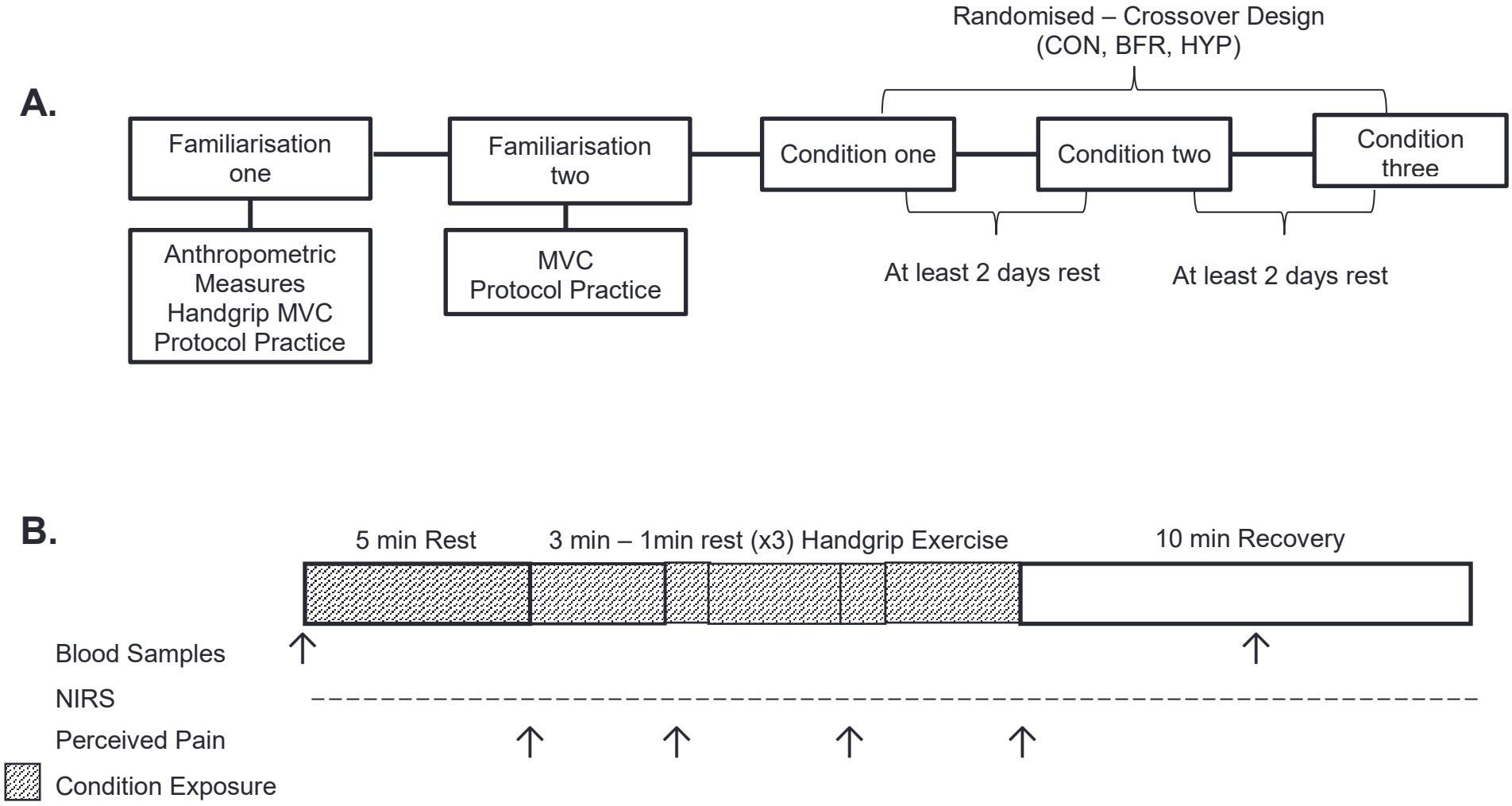

Figure 3.1. Schematics detailing: A. experimental overview and B. experimental protocol procedures.

3.6 Handgrip Exercise Protocol

The handgrip system used previously (see section 3.4) was utilised for the exercise protocol. Participants exercised in a supine position with the same randomised arm placed in a custom made rest with a 10° incline to standardise the arm position and encourage drainage. Exercise consisted of contracting the forearm at 60% MVC at a rate of one contraction (0.5 second) every 4 seconds for 3 minutes (1 set = 45 reps), participants completed three sets with a minute rest in-between each. Participants monitored the frequency and intensity of their handgrip contractions on a digital display using Lab Chart 6 (AD Instruments, Australia) (Figure 3.2) and when required the researcher provided verbal cues to ensure the participant adhered to exercise intensity. Exercise intensity was then later analysed to ensure work load was reproducible between experimental conditions (section 4.1). This exercise protocol was employed as previous work (Credeur et al. (2010) demonstrated that this exercise protocol increased handgrip MVC over a four week training regime in both a control group and BFR group.

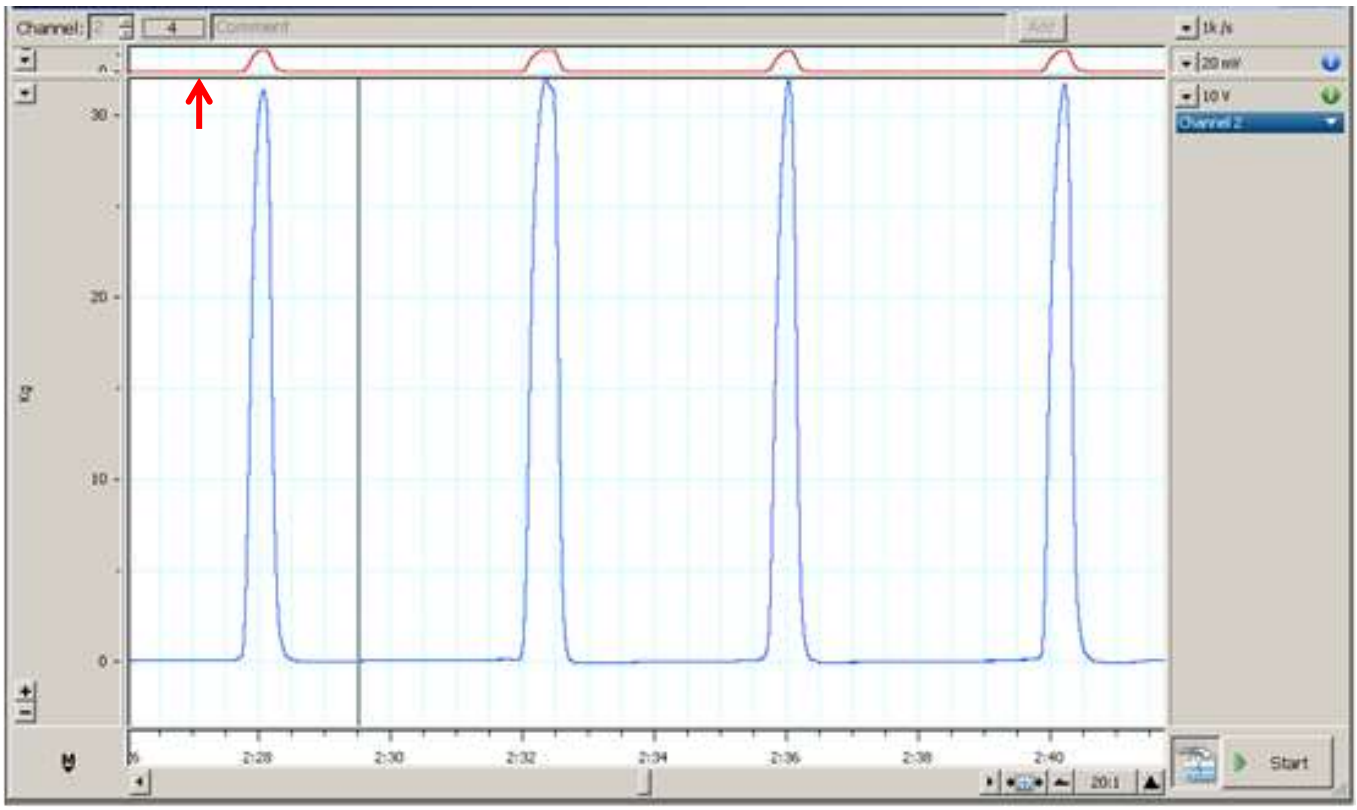

Figure 3.2. Screen shot of Lab Chart 6 live output used by participants to maintain cadence and exercise intensity throughout. ↑ = 60% MVC.

3.7 Blood Flow Restriction Condition

Venous occlusion was achieved through the inflation of a segmental pneumatic 11 cm wide cuff (SC10^{TM}, Hokanson, USA) to 80 mmHg via an automated cuff inflator system (E20 Rapid Cuff Inflator, Hokanson, USA) and compressed air source (AG101, Hokanson, USA).This pressure was previously used by Credeur *et al* (2010) and represents average diastolic blood pressure. Loenneke et al. (2013) recommended the use of a wider cuff to ensure participant comfort and induce optimal venous pooling. The cuff was placed 5 cm proximal to the antecubital fossa (Figure 3.3) and was inflated 5 minutes prior, and throughout the handgrip exercise including between set rests. Total cuff inflation time was 17 minutes.

Figure 3.3. Experimental arm during blood flow restriction (BFR) condition.

3.8 Hypoxic Exposure Condition

Participants breathed normobaric hypoxic air (14% O_2) at a rate of 7600 L.hr through a fitted mask via the Everest Summit II Generator (Hypoxico, The Altitude Centre, USA). Participants were exposed to the condition 5 minutes prior, and throughout the handgrip exercise.

3.9 Assessment of Skeletal Muscle Oxygenation

Near-infrared spectroscopy (NIRS) is a non-invasive optical technique widely used to monitor skeletal muscle oxygen content *in situ (Pereira et al., 2007)*. Haemoglobin (Hb), myoglobin (Mb) and cytochrome oxidase absorb light photons in the 700 -1300 nm spectrum, although Mb only represents a small proportion of the NIRS signal thus Hb is considered the main light absorbing chromophore (Mancini et al., 1994). Specifically peak absorption of O_2Hb occurs at 850 nm and HHb at 760 nm with the sum difference between the two providing an index of relative change in total haemoglobin (THb) (Pereira et al., 2007). Skeletal muscle is a non-uniform scattering medium therefore NIRS uses the modified Lambert-Beer law to calculate the absorption of the chromophore (O_2Hb, HHb):

$$OD_\lambda = \varepsilon_\lambda .c.L.DPF + OD_{R\lambda}$$

Where OD_λ is the optical density of the medium (skeletal muscle), ε_λ is the chromophore extinction coefficient ($\mu M^{-1}.cm^{-1}$), *c* the concentration of the chromophore (µM), *L* the distance between light entry, *DPF* the differential path-length factor and exit (cm), λ the wavelength used (nm) and $OD_{R,\lambda}$ the oxygen independent loss optical loss which allows the calculation of change in oxygen concentration:

$$\Delta c = \frac{\Delta OD\lambda}{\varepsilon\lambda . L . DPF}$$

NIRS has been used vastly to investigate skeletal muscle and cerebral oxygen content due to its high correlation with venous oxygen saturation (Mancini et al., 1994). Various groups have documented the reproducibility of NIRS in skeletal muscle oxygenation during resistance exercise, with van Beekvelt et al. (2002)

demonstrating a CV of 16-23% at various handgrip intensities, Tanimoto and Ishii (2006) observing a reproducibility coefficient of 0.85 during isotonic knee extensions and Pereira et al. (2005) also demonstration high reproducibility in vastus lateralis oxygenation (Pereira et al., 2007).

A portable NIRS device (Portamon MK II, Artinis Medical Systems) a wireless two-wavelength continuous system specially designed for skeletal muscle was used in this study. The device (83.8 by 42.9 by 17.2 mm) was placed upon the flexor digitorum superficialis muscle and affixed with a 13 cm wide elastic black cuff to prevent external light absorption. To enable accurate repositioning of the device, measurements were made and recorded pursuant to the lateral epicondyle of the humorous. Changes in O_2Hb, HHb and THb were measured through changes in absorption characteristics at 760 and 850 nm; additionally, a Total Saturation Index (TSI) was computed by O_2Hb – HHb. The device transmits light from three transmitters distanced 30, 35 and 40 mm away from the receiver and a DPF of 4.0 was selected due to previous reporting's (van Beekvelt et al., 2002) and manufacturers recommendations. In a similar manner to Buchheit et al. (2011) prior to the experimental procedure the NIRS device measured skeletal muscle haemoglobin variables for 2 minutes thus enabling subsequent results to be reported as a change from baseline in micro molar (µM) units. Throughout the baseline measures and experimental procedure, the NIRS device was connected to a personal computer for live data acquisition via Bluetooth at a sample rate of 1Hz. Haemoglobin variables and TSI were reported as a Δ from baseline, with each time point being averaged for statistical analysis.

An additional six participants (age 22.1 ± 0.8 y, height 175.5 ± 6.4 cm, mass 75.4 ± 8.9 kg) completed the CON condition for test-retest reliability of the NIRS derived variables (Section 4, Table 4.2). Six participants were used due to only having the NIRS device on loan for a short period of time.

3.10 Blood Collection

A trained phlebotomist withdrew blood samples using a safety butterfly blood collection system (25G x ¾", Greiner Bio-One, UK) from the antecubital region utilising standardised venepuncture techniques. Blood samples were obtained at baseline and 5 minutes' post exercise into vacuette containers containing 3.2% sodium citrate (Vacuette®, Greiner Bio-One, UK), samples were inverted immediately to cause anti-coagulation.

3.11 Sodium Citrate Treated Blood

Blood was immediately processed for glutathione status, 2 ml whole blood was pipetted into a 50 ml centrifuge tube onto 8 ml of 5% (w/v) Metaphosphoric acid (Sigma-Aldrich, UK), mixed thoroughly via vortex (Vortex Mixer, Phillip Harris, UK) to destroy red blood cells/enzymatic activity and kept on ice for 15 minutes. Subsequently, 1.5 ml was pipetted twice into two eppendorf tubes and centrifuged at 3,000 rpm at 4 °C for 15 minutes, clear supernatant pipetted into two new eppendorf tubes and placed into a -80 °C freezer until future analysis.

3.12 Glutathione Analysis

A commercially available kit was utilised to quantify both TGSH and GSSG (Glutathione Detection Kit, ADI-900-160, Enzo Life Sciences, UK) via an enzymatic recycling reaction. A standard curve was created by serially diluting 4 µM GSSG with 50 µL of assay buffer at four concentrations (100, 50, 25, 12.5 pmol) in triplicate. Sodium citrate treated experimental samples were diluted 1:100 with assay buffer; 50 µL of each sample were added to a 96 well plate in duplicate. Glutathione Reductase was added to 5,5'-dithio-bis2-nitrobenzoic acid (DTNB) at a ratio of 1.25 µL: 1ml, with 150 µL added to each well to create 5-thio-2-nitrobenzoic acid (TNB). Following a 10 second orbital shake absorbance of TNB was measured every minute for 10 minutes at 405 nm in a micro-plate reader (Infinite F200® Pro, Tecan, Switzerland) using Magellen™ software (Magellen Tracker, Tecan, Switzerland).

GSSG was quantified in the same manner as TGSH however experimental samples and 4 µM GSSG standards were treated with 1 µL of 2M 4-vinylpyridine (Sigma-Aldrich, UK) to prevent free thiols cycling. A one-hour incubation period at room temperature in the absence of light followed. Experimental samples were serially diluted 1:50 with assay buffer and the TGSH procedure replicated.

The average of triplicate 4 µM GSSG standard and duplicate of experimental samples were plotted against absorbance (405 nm) enabling the slope to be determined for the linear portion of each curve with back ground absorbance subtracted. Net slopes of the 4 µM GSSG standards were plotted against pmoles of GSSG, experimental samples were then plotted against the standards using trend analysis of the linear curve (R^2 = 0.97) to quantify pmoles of both TGSH and GSSG. A Glutathione ratio was then calculated as GSSG:TGSH. The intra-plate CV was 3.7% for TGSH and 2.6% for GSSG.

3.13 Assessment of Perceived Pain

A visual analogue scale (VAS) (Figure 3.4) was used to determine acute pain during each exercise condition. This scale has been used extensively within the literature to assess pain during this type of exercise (Borg, 1998, Manimmanakorn et al., 2013a). Participants were shown the VAS 30 seconds prior to end of the 5 minute rest and each exercise set and asked to communicate a score.

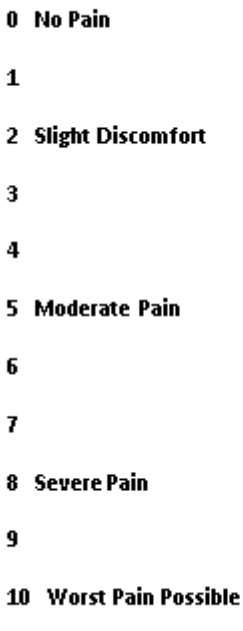

Figure 3.4. Visual analogue scale to monitor perceived pain during exercise.

3.14 Statistical Analysis

To decrease the likelihood of a type II error a *priori* power calculation was performed (G*Power 3, Germany), with an α value of 0.05 and a power of 99% eight participants (n = 8) were required in line with a previous BFR tissue oxygenation study (Karabulut et al., 2014).

Statistical analysis was performed using linear mixed models (SPSS v.21, IBM, USA) to analyse mean differences in all variables between the three conditions (CON, BFR, HYP). When significance was present, Sidak post-hoc analysis tests were used to locate significant pairs. Two-tailed significance was accepted at an α value of p = 0.05 and when significance was present 95% confidence intervals (CI) were reported.

Reproducibility statistics were reported for exercise intensity in the manner of mean change using the linear mixed model reported above, coefficient of variation (CV) and typical error (TE) using Excel 2010 (Microsoft, USA). NIRS haemoglobin variables were subject to test-retest reliability in the control condition (n=6) and presented as TE and CV.

3.15 Skill Acquisition and Student Journey

The protocol outlined in the method section required the formation of various collaborations outside the University of Bedfordshire due to limited experience of BFR exercise research within the initial supervisory team. One supervisor had previously supervised an ischemic pre-conditioning study.

Initially a visit was made to St Mary's University to meet Dr Stephen Patterson to discuss a research question presented by the initial supervisors, this was related to finding an 'optimal' restriction pressure for both the upper and lower limbs to use during BFR exercise. The visit to St Mary's provided an opportunity to ask questions and discuss BFR with somebody who had completed multiple studies in the area and test equipment that the laboratory had.

Following the visit to St Mary's University a purchase was made of the equipment their laboratory had to restrict blood flow, which was being used throughout the literature (section 3.7). The author developed skills using Doppler ultrasound with the help of Dr Jo Richards (University of Bedfordshire) to effectively measure blood flow in the brachial artery whilst implementing a ramped protocol to inflate a restriction cuff. This allowed the quantification of pressure which is required to restrict blood flow to the arm during rest. Through pilot work it was noted that upper limb restriction pressure was exactly the same as systolic blood pressure, this was the primary reason for the change of direction. Additionally, this was currently being investigated extensively by Dr Jeremy Loenneke in the United States, both in the lower and upper limbs. It was evident that the author could not recruit participants to a similar level or provide any additional insight to the area.

It was evident that there were gaps in the literature of BFR exercise, especially with regards to skeletal muscle oxygenation and oxidative stress. The author developed the current research question and method, presenting it to the initial supervisors who agreed the change in direction. New research ethics were written and submitted.

The University of Bedfordshire did not have a devise to measure muscle oxygenation, therefore a collaboration was formed with Dr Phillip Hennis at University College London's Institute for Sport, Exercise and Health (ISEH). Dr

Phillip Hennis visited the University of Bedfordshire where the author presented his proposed study, following this an agreement was made to borrow the Institute's NIRS device. The author arranged insurance cover for the costly NIRS device and visited the ISEH laboratories to collect. Further advice was sought from Dr Benjamin Jones on the use and analysis of NIRS data during a visit to the University of Essex.

Circumstantially, the author had to acquire the wet lab skills to perform the blood analysis by himself, therefore assistance was provided by two other students at the University of Bedfordshire (Josh Foster and James Tuttle) to help him through the process. Multiple ELISA kits were used to practice and determine the dilution factor required for the samples to fall within the standard curve. Dr John Hough at the University of Bedfordshire was able to teach the author and fellow student to use the plate reader software, which was then set-up and programmed by the author. The author trained laboratory technician Callum Mould to process the blood after collection for freezing.

The NIRS TSI data from the current investigation was presented at the American College of Sports Medicine annual conference by one of the authors initial supervisors, in the form of a poster presentation and abstract (both written by the author).

CHAPTER 4: Results

4.1 Exercise Intensity

There was no difference between conditions for exercise intensity ($F_{2,69}$ = 1.11, p = 0.337). Additionally, the reproducibility statistics demonstrate high reproducibility between each of the conditions (Table 4.1).

Table 4.1. Participant exercise intensity and between conditions reproducibility.

	Exercise Intensity (% MVC)			
Condition	**Set 1**	**Set 2**	**Set 3**	**Total**
CON	59.4 ± 2.5	58.9 ± 2.4	59.3 ± 3.5	59.2 ± 2.7
BFR	60.6 ± 1.0	59.7 ± 1.0	60.1 ± 1.8	60.2 ± 1.3
HYP	59.8 ± 2.5	59.7 ± 3.3	60.1 ± 2.0	59.9 ± 2.5
	P value	**CV (%)**	**TE (%)**	
CON - BFR	0.39	3.1	1.82	
CON - HYP	0.67	4.9	2.86	
BFR - HYP	0.96	3.2	1.88	

Data presented as mean ± SD. CV = coefficient of variation, TE = typical error. No significant difference between conditions.

4.2 Tissue Oxygenation Variables

4.2.1 Tissue Oxygenation Reliability

The test-retest reliability of the NIRS derived tissue oxygenation variables was considered low. The mean CV for all sets combined was as follows: TSI 4.0%, O_2Hb 3.8%, HHb 4.2% and THb 3.9%. The TE and CV for all individual sets and total are presented below (Table 4.2).

Table 4.2 Test - retest reliability statistics for NIRS derived haemoglobin variables in the CON condition (n = 6).

	Pre		Set 1		Rest 1		Set 2		Rest 2		Set 3		Recovery		Total	
	CV (%)	**TE**	**CV (%)**	**TE**	**CV (%)**	**TE**	**CV**	**TE**	**CV (%)**	**TE**	**CV (%)**	**TE**	**CV (%)**	**TE**	**CV (%)**	**TE**
TSI (%)	7	0.9	1.4	2.8	0.9	1.0	12.8	2.2	1.1	1.1	2.3	1.9	2.2	2.0	4.0	1.7
O_2Hb (µmol)	8.5	2.1	1.3	5.8	3.4	5.9	1.8	6.5	3.2	5.5	2.2	6.9	5.9	4.2	3.8	5.3
HHb (µmol)	2.5	1.7	5.3	6.9	1.8	2.5	5.3	3.8	1.8	2.5	9.3	7.9	3.2	4.9	4.2	4.3
THb (µmol)	1.1	1.0	1.3	4.1	2.4	4.8	1.4	4.1	2.3	3.4	1.3	3.1	3.0	6.7	1.8	3.9

CV = coefficient of variation, TE = typical error

4.2.2 Typical Muscle Oxygenation

Typical muscle oxygenation response of the forearm flexors to the exercise intervention in all conditions at a rate of 1Hz (Figure 4.1).

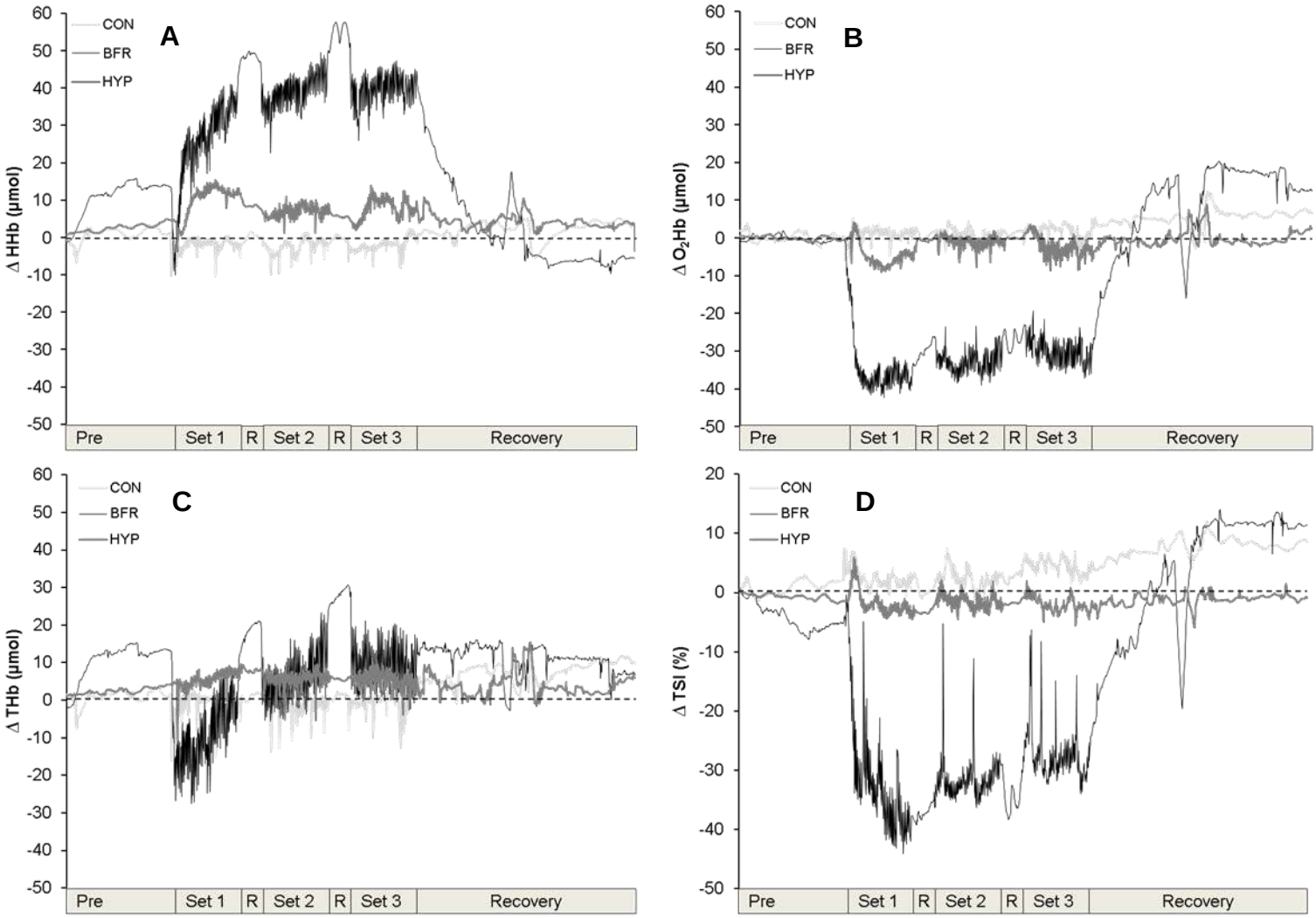

Figure 4.1. Typical individual response showing changes in **A.** HHB, **B.** O_2Hb, **C.** THb, **D.** TSI from a pre exercise calibration period at a sample rate of 1Hz.

4.2.3 Total Saturation Index

There was a significant main effect for condition in mean TSI ($F_{2,21}$ = 23.12, p = 0.007) between CON (-1.3 ± 6.8 %), BFR (-11.5 ± 10.3%) and HYP (-4.5 ± 5.1 %). On average, TSI was 10.2% lower in the BFR condition compared to CON (p = 0.007, 95% CI -17.9 to -2.6 %), however there was no significant difference between any other conditions (p > 0.05).

A significant main effect for time was present, with TSI decreasing ($F_{6,126}$ = 12.41, p < 0.001). A significant condition x time interaction effect ($F_{6,126}$ = 2.39, p = 0.008) took place with multiple individual time point interactions (Figure 4.2).

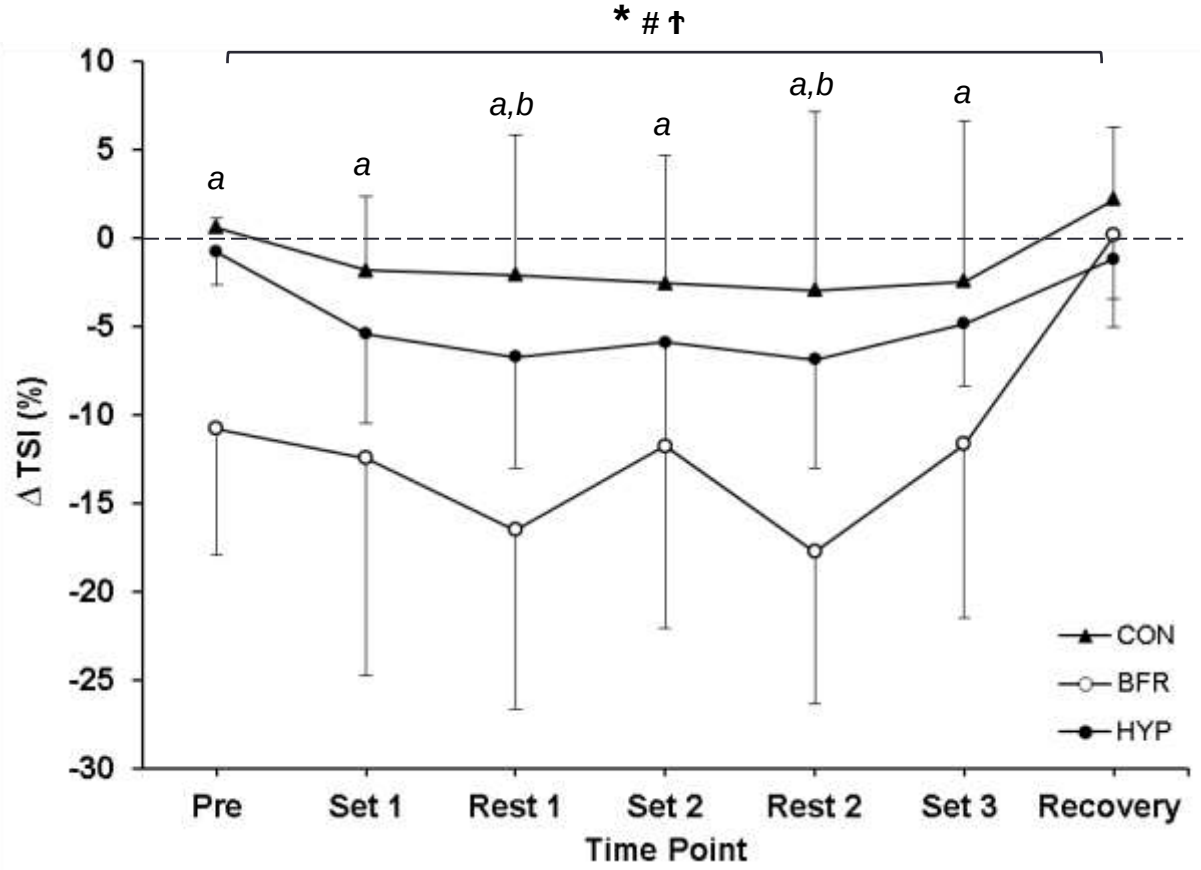

Figure 4.2. Mean change from resting in TSI between conditions (CON, BFR, HYP) across all time points. Values presented as mean ± SD. * Denotes significant difference between conditions, # significant difference over time † significant condition x time interaction, individual time point interactions *a* = CON - BFR and *b* = BFR - HYP (p < 0.05).

4.2.4 Oxygenated Haemoglobin

There was no difference in O_2Hb between conditions, ($F_{2,18}$ = 0.18, p = 0.84) in CON (-3.28 ± 8.90 µmol), BFR (-4.99 ± 10.06 µmol) or HYP (-5.23 ± 7.61 µmol). There was however, a significant main effect for time ($F_{6,126}$ = 12.14, p < 0.001) with O_2Hb decreasing over time. There was no significant interaction effect ($F_{12,126}$ = 0.52, p = 0.995) (Figure 4.3).

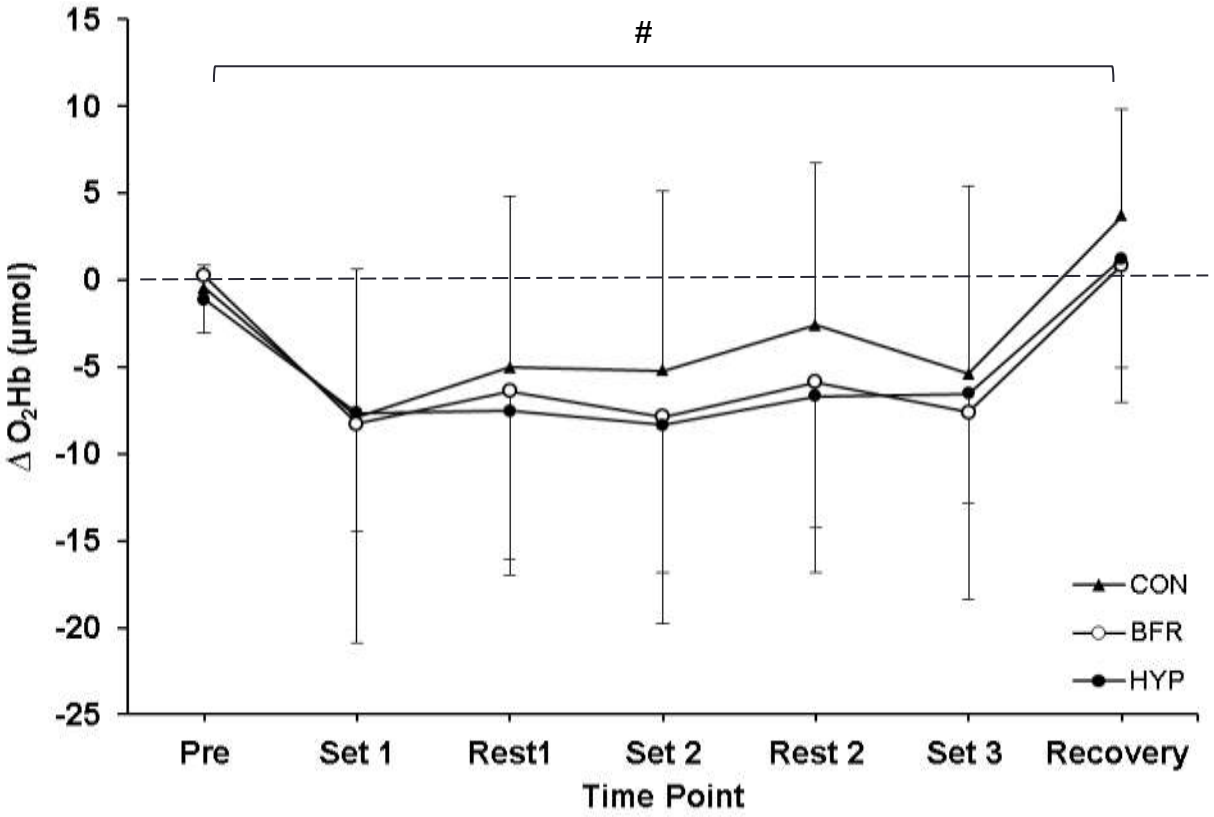

Figure 4.3. Mean change from resting in O_2Hb concentrations between conditions (CON, BFR, HYP) across all time points. Values presented as mean ± SD. # significant main effect for time (p < 0.05).

4.2.5 De-oxygenated Haemoglobin

There was a significant main effect of condition for HHb between ($F_{2,21}$ = 9.70, p <0.001), in CON (2.06 ± 5.87 µmol), BFR (14.39 ± 11.96 µmol) and HYP (6.83 ± 6.11 µmol). In the BFR condition HHb was 12.33 µmol higher compared to CON (p < 0.001, 95% CI 5.01 to 19.66 µmol) and 7.56 µmol higher compared to HYP (p = 0.042, 95% CI 0.23 to 14.89 µmol) respectively, with no difference between CON and HYP (p > 0.05).

During the protocol HHb increased significantly over time ($F_{6,126}$ = 19.33, p < 0.001), and there was a significant main interaction effect ($F_{2,126}$ = 5.66, p < 0.001). The BFR condition presents multiple individual time point differences with HHb being significantly higher, further enhanced by rest (Figure 4.4).

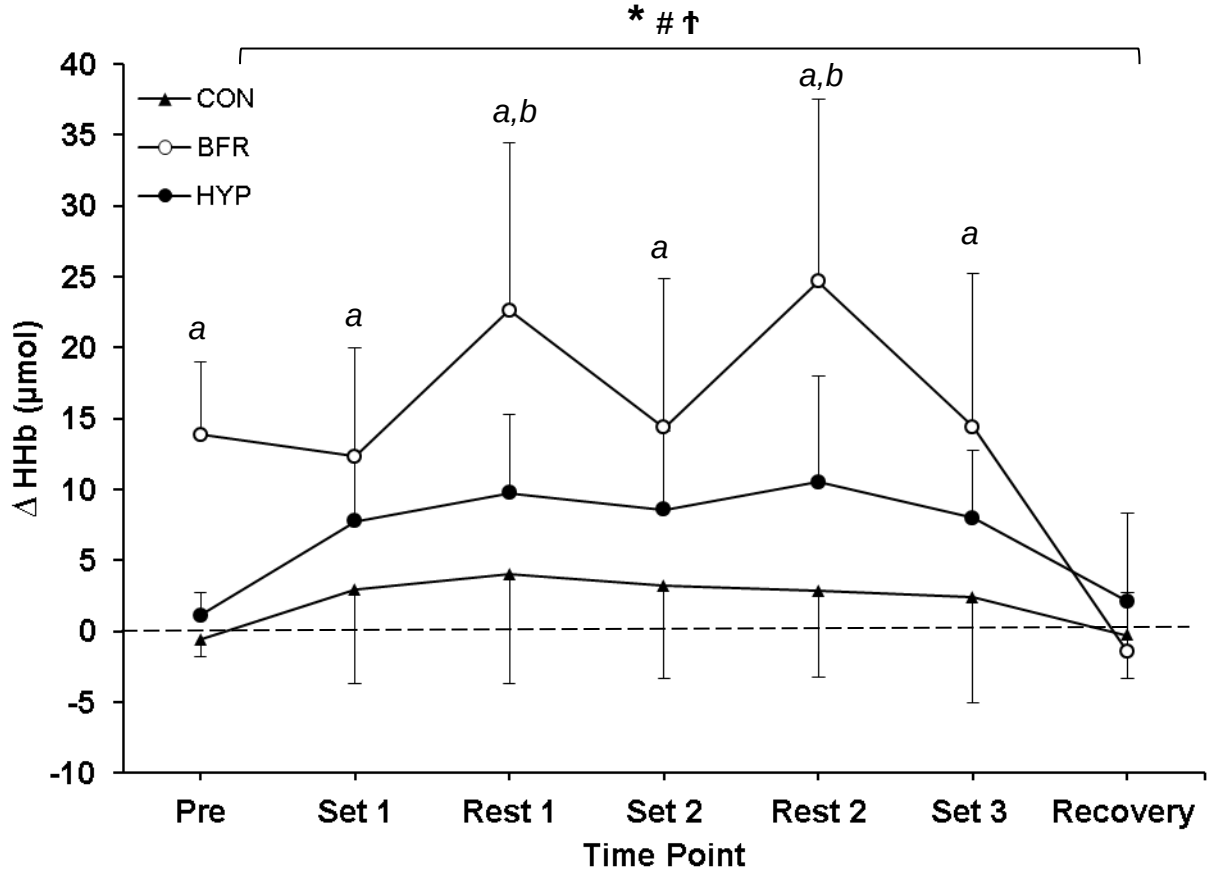

Figure 4.4. Mean change from resting in HHb concentrations between conditions (CON, BFR, HYP) across all time points. Values presented as mean ± SD. * Denotes significant difference between conditions, # significant difference over time † significant condition x time interaction, individual time point interactions *a* = CON - BFR and *b* = BFR – HYP (p < 0.05).

4.2.6 Total Haemoglobin

There was a significant main effect of condition in THb concentration ($F_{2,21}$ = 11.53, p < 0.001), during CON (-1.22 ± 5.50 µmol), BFR (9.41 ± 9.54 µmol) and HYP (1.59 ± 5.04 µmol). In the BFR condition THb was 10.60 µmol higher compared to the CON condition (p <0.001, 95% CI 4.68 to 16.57 µmol) and 7.82 µmol higher compared to the HYP condition (p = 0.008, 95% CI 1.86 to 13.75). There was no difference between CON and HYP conditions (p > 0.05).

A significant main effect for time was present ($F_{6,126}$ = 8.30, p < 0.001), with THb increasing significantly during rest periods and decreasing during exercise. Additionally, a significant main interaction effect was present ($F_{12,126}$ = 6.63, p < 0.001) and multiple individual time point differences between conditions (Figure 4.5).

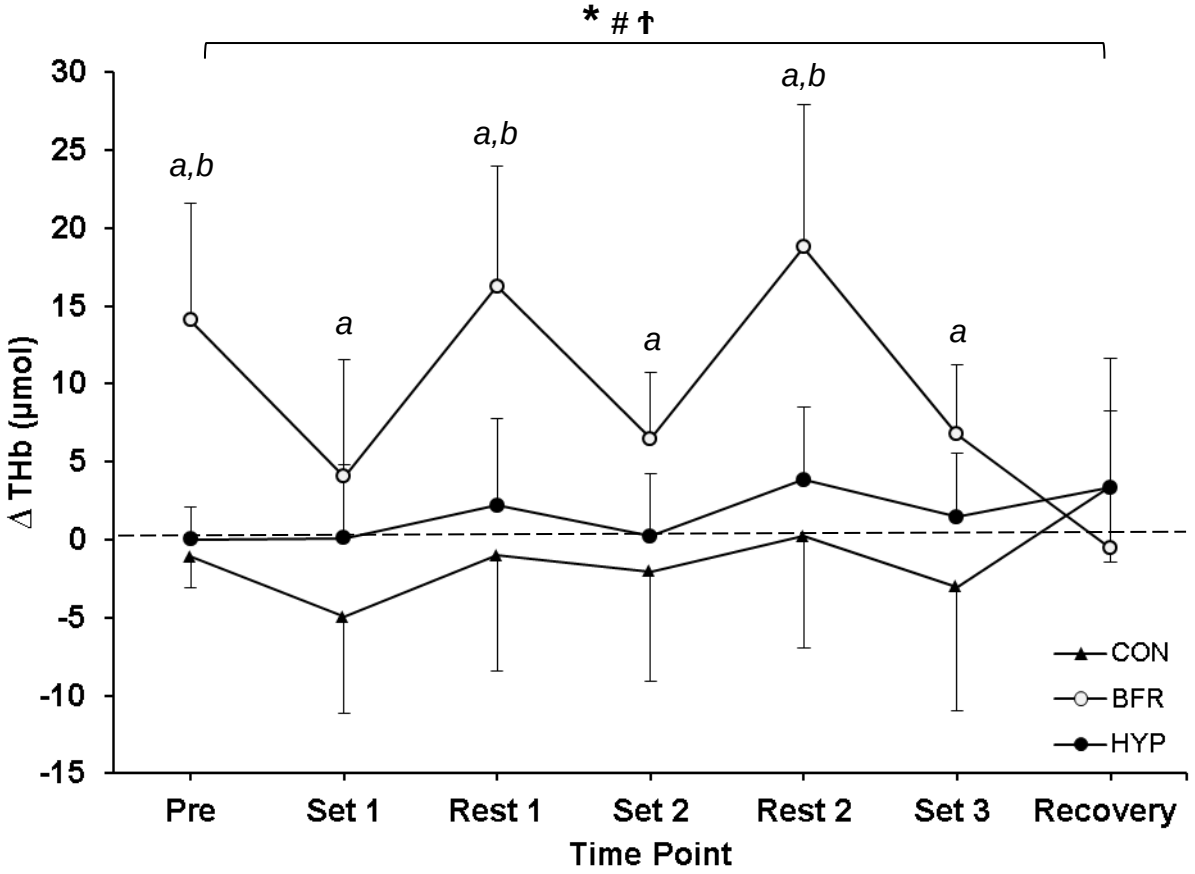

Figure 4.5. Mean change from resting in THb concentrations between conditions (CON, BFR, HYP) across all time points. Values presented as mean ± SD. * Denotes significant difference between conditions, # significant difference over time † significant condition x time interaction, individual time point interactions *a* = CON - BFR and *b* = BFR – HYP (p < 0.05).

4.3 Whole Blood Glutathione

4.3.1 Whole Blood Glutathione Standard Curves

Standard curves were produced to enable individual samples to be plotted using trend analysis of the linear curve (Figure 4.6 & 4.7).

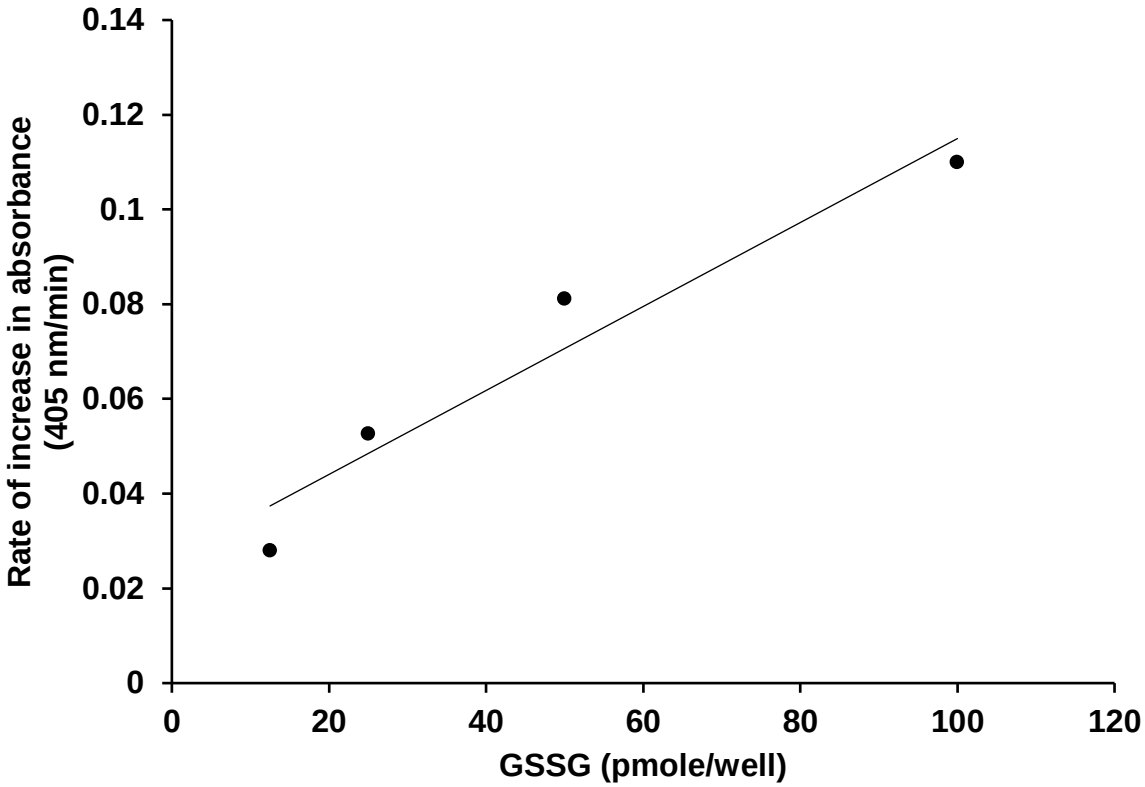

Figure 4.6. Linear absorbance curve of the GSSG standards with background absorbance subtracted.

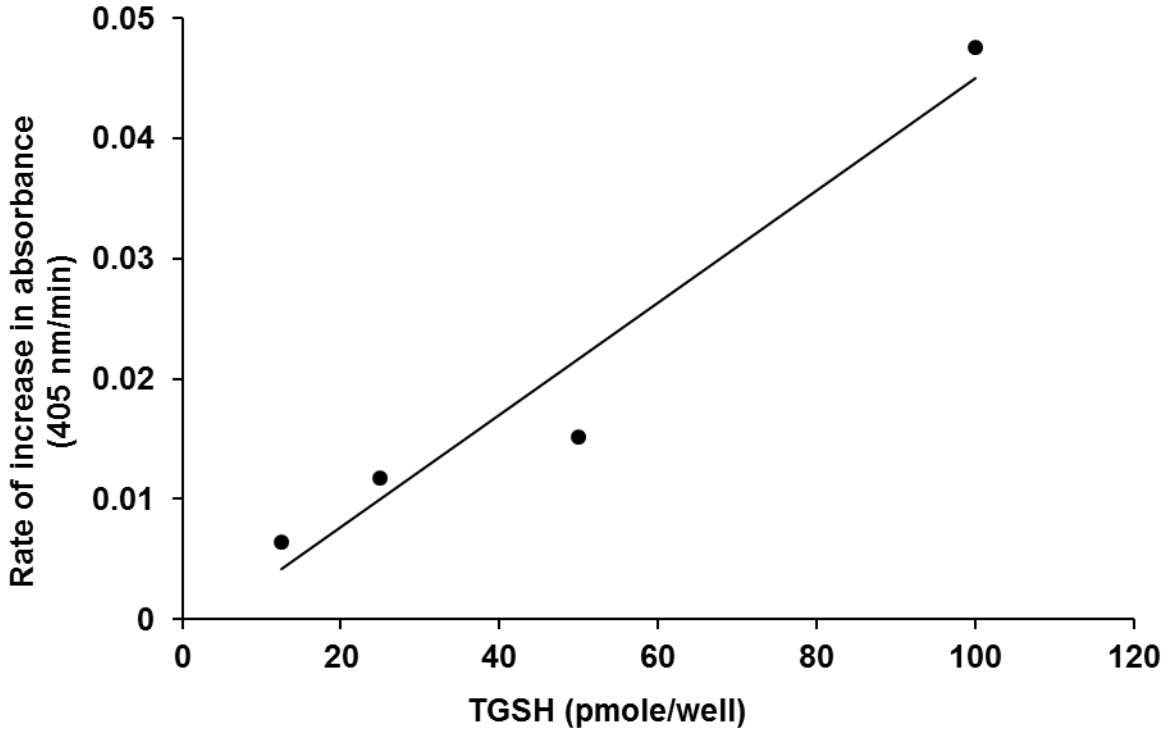

Figure 4.7. Linear absorbance curve of the TGSH standards with background absorbance subtracted.

4.3.2 Whole Blood Glutathione Ratio

There was no difference between condition ($F_{2,15}$ = 2.92, p = 0.085), across time ($F_{1,15}$ = 0.91, p = 0.355) or a condition x time interaction effect ($F_{2,15}$ = 1.02, p = 0.383) for circulatory GSSG/TGSH ratio (Figure 4.8).

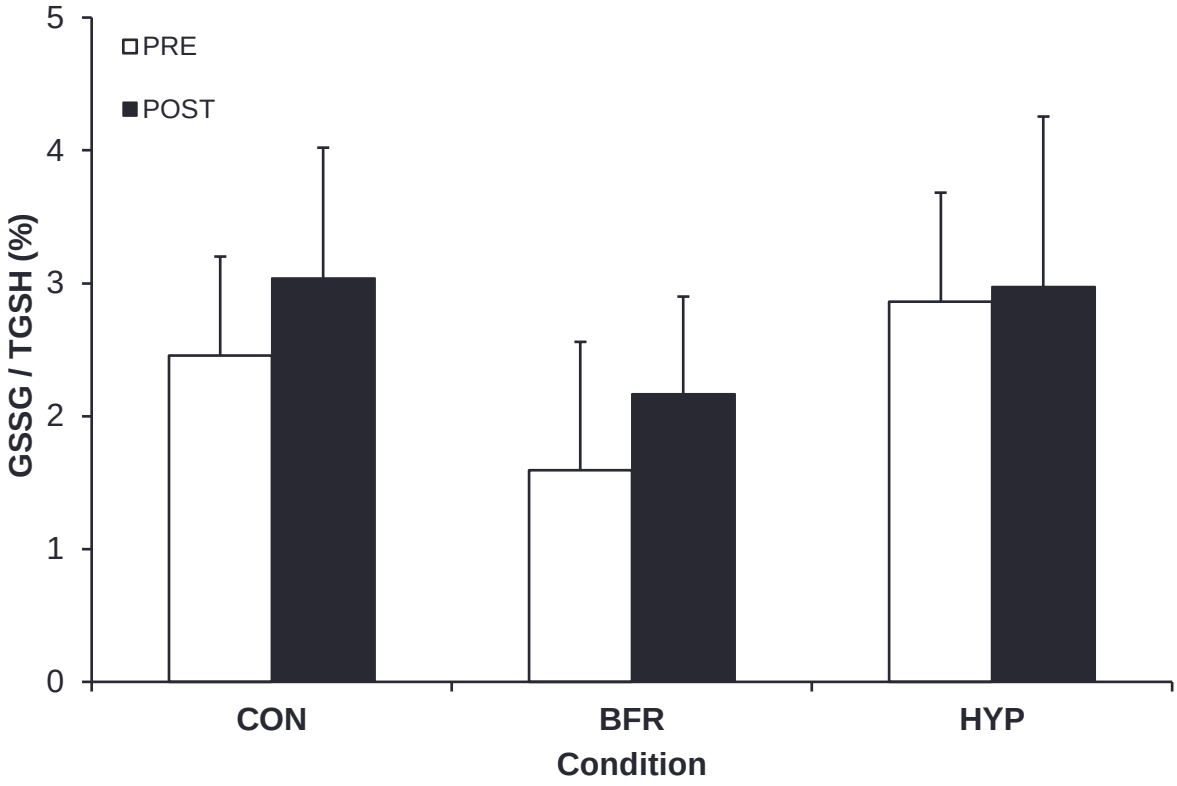

Figure 4.8. Mean change in whole blood glutathione status pre – post exercise [ratio of oxidised glutathione (GSSG) to total glutathione (TGSH)] in all conditions (CON, BFR, HYP). Values presented as mean ± SD.

4.4 Visual Analogue Scale Perceived Pain

A significant main effect for condition was present ($F_{2,67}$ = 338.96, $p < 0.001$), with BFR (6 ± 1 a.u.) condition having higher perceived pain than both CON (2 ± 2 a.u.) and HYP (2 ± 2 a.u.) ($p < 0.01$), however there was no difference between CON and HYP (Figure 4.9).

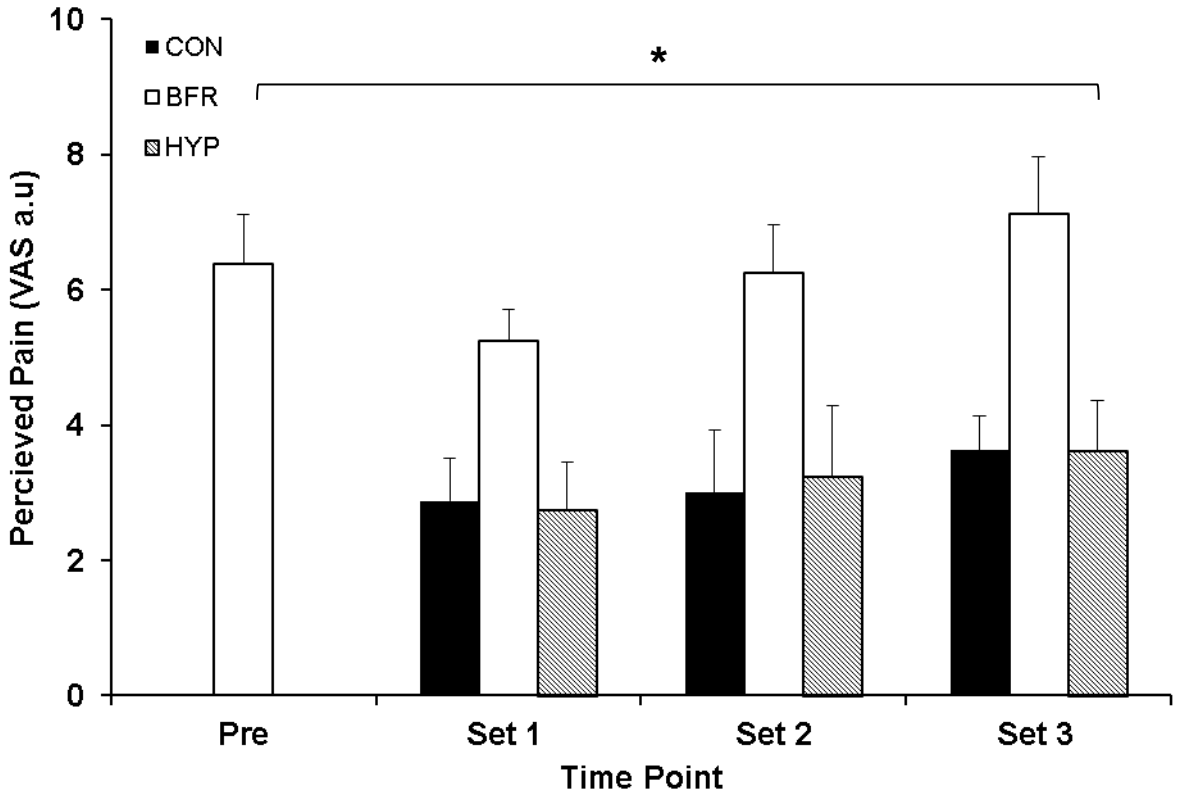

Figure 4.9. Mean change in perceived pain between conditions (CON, BFR, HYP) across all time points. Values presented as mean ± SD. * Denotes significant difference between conditions, with BFR being significantly higher than both CON and HYP ($p < 0.05$). CON and HYP = 0 for pre.

CHAPTER 5: General Discussion and Conclusions

The purpose of the present study was to investigate whether the addition of BFR or HYP to handgrip RE would modify skeletal muscle tissue oxygenation, oxidative stress response (GSSG:TGSH) and the perceived pain that these novel exercise modalities might elicit.

The current findings were partially in agreement with the first hypothesis, whereby the addition of BFR during RE significantly increased THb and HHb whilst lowering TSI in skeletal muscle compared to both CON and HYP before and throughout the exercise protocol, however there was not difference in O_2Hb.

The second hypothesis was accepted due to HYP exerting no additional effect on skeletal muscle oxygenation (TSI, O_2Hb, THb, HHb) before, during or after exercise compared to CON.

Moderate intensity handgrip exercise (CON) did not result in oxidative stress, neither did the addition of BFR or HYP when oxidative stress was measured by GSSG:TGSH, rendering the third hypothesis null.

Finally, the fourth hypothesis was accepted whereby the addition of BFR to RE was perceived to be significantly more painful compared too CON and HYP. The addition of HYP to RE had no effect on PP.

5.1 Tissue Oxygenation

Firstly, it should be noted that the use of continuous wave NIRS (cw-NIRS) during this study does not reflect the true absolute value of muscle oxygenation, however reflects a change from baseline values (Pereira et al., 2007). Adjustments for oscillations as a result of muscle stretching were not required, due to each exercise condition completing the same number of repetitions (Larkin et al., 2012) Absolute values can be obtained via arterial occlusion techniques to provide measurements of mitochondrial oxygen consumption, providing information on muscle oxygen consumption and recovery kinetics (Jones et al., 2015), the present study did not use these techniques.

5.1.1 Blood Flow Restriction

The present investigation has demonstrated an increase of 10.60 µmol in blood volume as measured by THb with the addition of BFR to RE, alongside an increase in HHb of 12.33 µmol when both compared to control. Larkin et al. (2012) reported similar findings in the vastus lateralis during low intensity (40 % 1RM, 10 sets, 12 reps, 1 min rest) RE, in a similar manner to the present study cw-NIRS was used however results were average for the whole exercise bout including rests, unlike the present study which averaged each set and rest. Larkin et al. (2012) reported an average BFR THb of 14.4 ± 1.6 µmol.cm compared to control 0.9 ± 1.6 µmol.cm and a BFR HHb 11.0 ± 2.5 µmol.cm with control (0.5 ± 1.1 µmol.cm). This increase in blood volume Larkin et al. (2012) observed coincided with post exercise increase in vascular endothelial growth factor (VEGF), the author contributed this angiogenic effect to a possible increase in shear stress applied to the vessel walls caused by the raised blood volume. Larkin et al. (2012) could not decipher whether the increase in blood volume during BFR RE is due to the exercise or the rest periods, this is because the group averaged the NIRS data for the whole RE regime. Hunt et al. (2013) showed that low intensity lower limb BFR training caused increased calf filtration and that this may indicate an increased capillarisation, therefore this could indicate that tissue oxygenation response may change with training, the current study only looked at the acute response.

The current study averaged the NIRS data for each time point, from this it was determined both THb and HHb increase significantly compared to CON during rest periods. A decrease is seen during handgrip exercise but still remains higher than CON, this could be attributed to the increase in THb and HHb in the previous rest period. Ganesan et al. (2015) reported similar kinetics in blood volume variables however this was with fatiguing moderate intensity knee extensions. The addition of BFR increased THb significantly during rest periods compared to repetition matched control, subsequently THb was higher during the two exercise sets following (Ganesan et al., 2015). The present investigation implemented a 5 minutes BFR period before the RE began this caused a significant increase in HHb (13.9 ± 5.1 µmol) compared to CON (-0.6 ± 1.1 µmol) and HYP (1.1 ± 1.5 µmol). This pre exercise BFR period could explain the increased HHb and THb during handgrip set

one, Ganesan et al. (2015) did not implement a pre exercise BFR period and saw no difference in HHb and THb during the first set of exercise in their study.

Cayot et al. (2014) observed a similar effect of the application of BFR before exercise commencement, with increases in HHb significantly higher compared to the BFR application immediately before and therefore the primary cause of increased blood volume could be explained by inter-set rest periods and pre BFR, therefore this should be a primary consideration when designing BFR protocols. The current investigations saw blood volume increases with rest periods of 60 seconds. No significant differences were present in O_2Hb during exercise or rest between conditions, this is due to arterial inflow being maintained with a cuff pressure (80mmHg) below systolic blood pressure Another consideration should be starting restriction pressure, Karabulut et al. (2014) saw greater increases in HHb with a higher initial restriction pressure.

Although there was clearly an increased blood volume in the present investigation (THb and HHb) with the application of BFR, only a speculation can be made on the intramuscular metabolic stress. However, an increase in blood volume supports the potential mechanism of cellular swelling, which has been associated with increased MPS (Keller et al., 2003). It is believed that the inflow of blood into intracellular space can enhance anabolic signalling through the disruption of cell membranes (Lang et al., 1998) and increase satellite cell mitosis (Dangott et al., 2000). Cayot et al. (2014) however, raise the concern that the increase in blood volume could be a result of blood accumulation in vessels/capillaries which were not recruited prior to the occlusion via muscle contraction or BFR.

5.1.2 Hypoxia

In line with the hypothesis there was no change in tissue oxygenation variables compared to CON, this was expected because when SpO_2 decreases compensatory vasodilation takes place to preserve muscle oxygenation (Casey and Joyner, 2012). The compensatory vasodilation during exercise induced hypoxia and systemic hypoxia reduces with ageing at handgrip intensities as low as 20%, a decrease in NO mediated dilation is the primary cause (Casey, 2011). It should also be

considered that with ageing or associated comorbidities such as chronic obstructive pulmonary disease, poor pulmonary perfusion results in lower levels of SpO_2 (Lynes and Kelly, 2009).

With this in mind Kon et al. (2012) saw SpO_2 values as low as 80% during moderate intensity (50% 1RM) RE in young healthy males. It could be postulated that in elderly participants this would drop even further. A limitation of the current study was that SpO2 was only used as an ethical safety consideration and not recorded, however during HYP or BFR SpO_2 never dropped below 90%. Scott et al. (2014) highlight that type II fibres are more sensitive to hypoxia mediated dilation compared to type I fibres (McDonough et al., 2005), with this increased O_2 supply they may act in a similar manner to type I fibres, thus reducing fatigue. Rates of fatigue during HYP resistance exercise have yet to be investigated.

5.1.3 NIRS Reliability

The current study saw high levels of reproducibility through low CV and TE for the NIRS derived variables (Table 4.2), however only six participants independent to the study completed the test-retest procedure. In addition, there is the possibility that levels of reproducibility could be affected by the exercise conditions. Utilising a similar NIRS method to the present study but in aerobic activity, Austin et al. (2005) found a test-retest CVs in the range of 7-11% for all Hb variables.

5.2 Oxidative Stress

The handgrip exercise protocol implemented in this study did not significantly increase pre-post RE oxidative stress when measured by GSSG:TGSH. Previous studies have reported oxidative stress following moderate/high intensity isometric handgrip protocols in the form of: increased LH (Alessio et al., 2000), decreased GSH (Steinberg et al., 2002) and increased TBARS (Alessio et al., 2000, Dousset et al., 2002). A potential explanation for the aforementioned responses could be that prolonged isometric contractions can decrease muscle blood flow and subsequently TSI (Fryer et al., 2014), this would cause an acute state of ischemia followed by reperfusion upon contraction release.

Following an isometric contraction (60% 1RM) to failure (42 ± 5 sec) Dousset et al. (2002) reported increased TBARS, during the contraction period the active muscle would be metabolically compromised (imbalance of ATP generation/degeneration and Ca^{2+} homeostasis) leading to an increase in xanthine oxidase (Bloomer and Goldfarb, 2004). Xanthine oxidase increases with RE (Spiering et al., 2008b, Ho et al., 2010) in a similar manner to ischemic reperfusion (McCord, 1985), when xanthine oxidase is inhibited exercise derived GSSG decreases (Vina et al., 2000), considering this Bloomer and Goldfarb (2004) postulate that xanthine oxidase is a key factor in ROS production following high intensity/exhaustive RE. However, Garten et al. (2015) reported xanthine oxidase to decrease following high intensity RE with and without BFR; showing an inverse relationship with oxidative stress (increased GSSG:TGSH and PC). The author believed that by altering the cuff during the BFR condition to maintain a SpO_2 of 95%, this decreased the hypoxic stimulus that converts xanthine dehydrogenase (XDH) into XO (Garten et al., 2015), however this does not explain why a decrease was present during high intensity RE without BFR. With the above in mind, the handgrip protocol utilised in this study was not to exhaustion and utilised rhythmic moderate intensity (60% 1RM) isotonic contractions, therefore it is likely that the level of metabolic compromise and ischemia experienced was not to the level of the isometric handgrip investigations mentioned which did elicit oxidative stress (Alessio et al., 2000, Steinberg et al., 2002, Dousset et al., 2002).

The current investigation could have benefited from the monitoring of BLa^-, it has been reported that increases in whole blood GSSG:TGSH correlate in a linear fashion to BLa^-/pyruvate ratio (Sastre et al., 1992). Previously both RE with BFR (Fujita et al., 2007, Pierce et al., 2006, Takano et al., 2005) and HYP (Kon et al., 2010, Kon et al., 2012) have demonstrated increased BLa^- compared to RE control, it would therefore be reasonable to think that the addition of BFR or HYP would increase GSSG:TGSH. Goldfarb et al. (2008) demonstrated the addition of BFR to low intensity RE to blunt the GSSG:TGSH and PC compared to without. In contrast Garten et al. (2015) found the addition of BFR to both high and low intensity RE did not alter GSSG:TGSH compared to matched control but did blunt PC, neither of these investigations reported BLa^- so a GSSG:TGSH relationship couldn't be observed.

It should be noted that previous investigations utilising GSSG:TGSH as a marker oxidative stress in BFR RE were in larger muscle groups compared to the present study where, Goldfarb et al. (2008) incorporated both bicep curls and calf extensions, with Garten et al. (2015) just bicep curls alone. The present study and those aforementioned all used GSSG:TGSH as a marker that reflects muscle morphology however it was measured in whole blood and not muscle (Garten et al., 2015). The presence of GSSG:TGSH in the blood does represent that of the muscle both at rest and during exercise (Veskoukis et al., 2009), what is not known is whether the outflow of GSSG:TGSH is effected by the increase in skeletal muscle blood volume (THb) that BFR elicits.

5.3 Perceived Pain

A decrease in handgrip strength with age coincides with an increased risk of mortality (Rantanen et al., 2003), however the maintenance and improvement of handgrip strength can ensure functional tasks of daily living can be undertaken (De Vreede et al., 2005). PP can be a barrier for exercise participation and completion, especially in the elderly (Song et al., 2003). The present findings suggest the addition of BFR to handgrip RE results in significantly more pain (6 ± 1 a.u), compared to both HYP (2 ± 2 a.u) and CON (2 ± 2 a.u), however this is only regarded as moderate pain. Wernbom et al. (2006) and Loenneke et al. (2011b) reported high acute pain responses to dynamic leg exercise to failure, even suggesting that pain would restrict BFR to only highly motivated individuals. It should be considered that both of these observations of increased PP were when exercise was to failure, however the present study utilised programme similar to a handgrip routine that has previously reported increases in strength (Credeur et al., 2010) and was not to failure. However, RE to failure only resulting in low levels of acute pain in the elderly (60 ± 2 y) have been detected, with PP decreasing with training (Valkeinen et al., 2006).

Healthy individuals were used for the current investigation and it is likely that PP would alter with ageing. An arm crank study has reported that PP actually decreases with age (Groslambert et al., 2006), however the ability to complete high intensity RE doesn’t (Frontera et al., 2000). The current study saw no difference in PP between

CON and HYP, however it is unknown whether the addition of HYP to the current handgrip protocol would increase muscular hypertrophy or strength to a greater degree than CON. Only one other study utilising HYP with RE has reported PP response, where PP was greater in HYP on days 4, 6 and 8 compared to both BFR and CON, however pain decreased with training and only ever reached a level of moderate pain (6 a.u) (Manimmanakorn et al., 2013a). These findings contrast the present findings which saw BFR to elicit a greater pain response, however Manimmanakorn et al. (2013a) exercised to failure rather than a set volume which could potentially explain the difference. The only previous research using set exercise volume (70% 1RM) saw increases in strength and Mcsa to a greater magnitude with HYP compared to CON (Nishimura et al., 2010), however the relatively high intensity would better suited to a trained population rather than elderly.

5.4 Conclusion

To conclude, the addition of BFR elicited increases in blood volume in the form of THb and HHb, this could potentially contribute to 'cellular swelling' which is understood to increase skeletal muscle hypertrophy. BFR also decreased TSI due to an increased ratio of HHb to O_2Hb, however there O_2 delivery doesn't change between conditions.

Moderate intensity rythmic isometric handgrip exercise alone, with BFR or HYP does not elicit a oxidative stress response when measured by whole blood GSSG:GTSH, however it cannot be ruled out that other stress markers may increase. This could be potentially beneficial for populations such as the elderly, where a compromised NO system is present.

PP can deter people from taking part or completing exercise, in the present study we concluded that the addition of BFR does increase PP compared to other RE modalities (HYP and CON), however this did not effect exercise completion as only 'moderate pain' was present.

Considerable research has taken place in recent years into the effects of BFR exercise, providing insight into both acute mechanisms and training response. However, little consideration has been taken to consider the general applicability of this novel exercise intervention. Past literature consistently suggests it's use in elderly populations and for injury rehabilitation, future research should transition from BFR primarily only being used as a research topic to developing and testing methods which can be used in every day life and whether they work.

CHAPTER 6: References

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CHAPTER 7: Appendices

Appendix A

DEPARTMENT OF SPORT &
EXERCISE SCIENCES

Bedford Campus
Polhill Avenue
Bedford
MK41 9EA

Participant Information Sheet

Localised and Systemic Hypoxia during Resistance Exercise

Dear Participant,

Thank you for showing an interest in participating in the present study. Please read this information sheet carefully before deciding whether to participate. If you decide to volunteer we thank you for your participation. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the aim of the project?

The aim of the study is to investigate whether blood flow restriction resistance exercise (the addition of an inflated cuff during exercise), resistance exercise in hypoxia (low levels of inspired oxygen) and resistance exercise alone cause oxidative stress. Oxidative stress occurs naturally in response to high intensity resistance exercise and is involved in the process of increasing muscle size (hypertrophy). In addition during exercise we aim to monitor the levels of oxygen in the exercising muscle.

What types of participants are needed?

Males between the ages of 18-26 are required for the study. Participants should be non-smokers, physically active and normotensive (normal blood pressure 120/80 mmHg).

What will be asked of participants?

Participants should refrain from dietary/vitamin supplementation (anti-oxidants, nitrates, protein, branch chain amino acids (BCAA's)), ergogenic aides/sports supplements (L-Arginine, β- Alanine, creatine) and exposure to extreme environments (high altitude, extreme heat, extreme cold).

Participants will be required to refrain from strenuous exercise, caffeine and alcohol 72 hours prior to experimental procedures

Participants will be asked to visit the laboratory on 5 different occasions:

Visit one – Familiarisation session one

Firstly you will fill out a blood analysis form and physical activity readiness questionnaire (PAR-Q), providing there are no contraindications procedures will precede. At this point you can ask any questions about the study. Height, mass and blood pressure will be measured in triplicate. You will be introduced to the handgrip exercise modality. This involves gripping a metal device which provides live feedback on grip strength. At this point you will perform three maximal voluntary contractions (MVC) with the handgrip device separated by one minute. From the produced MVC results you will practice rhythmic handgrip exercises at a mild intensity utilising a intensity feedback system.

Visit two – Familiarisation session two

- You will complete the procedures of familiarisation session one, minus the health parameters.

Visit three – Exercise condition one

- Upon arrival you will fill out a form to ensure you have adhered to pre-test exclusion criteria.
- You will lie down for 15 minutes before having a blood sample withdrawn via the venepuncture technique from the exercising arm (randomized).
- Following this you will have a small device attached to your forearm with strapping (NIRS); this will measure the oxygenation of your exercising muscle. The device emits an infra-red light and does not cause any discomfort or risk of injury.
- You will rest for 5 minutes under exposure to the experimental condition (described below), exposure to the condition will continue until the end of exercise.
- 3 sets of handgrip exercise (45 reps, medium intensity 60% MVC) will proceed with one minute rest in between sets.
- Upon completing the exercise you will rest for 5 minutes, at which point you will have another blood sample withdrawn.
- You will rest for 5 minutes more at which point you will be able to leave.

Visit four – Exercise condition two (48-72 hours after visit three)

- The procedure of visit three will be replicated under a different condition (described below).

Visit Five – Exercise condition three (48-72 hours after visit three)

- The procedure of visit three will be replicated under a different condition (described below).

Experimental Conditions –

Blood Flow Restriction –

- An inflatable cuff (similar to a blood pressure cuff) will be placed on the upper arm.
- The cuff will be inflated to a pressure of 80mmHg, this pressure will allow blood to flow into the arm but restrict some of the blood flow out.

Hypoxia –

- You will inhale low oxygen air through a mask, the level of oxygen will be 14% whereas, which is 7% lower than sea level oxygen (21%).

Control –

- This condition will be exercise only.

Potential risks posed to the participant.

1. Exposure to blood flow restriction
2. NIRS
3. Hypoxia
4. Handgrip exercise
5. Blood collection

How will these risks be minimized?

1. With blood flow restriction, there are risks of; heart attacks, bruising, and muscle damage in certain populations (elderly). To prevent these risks, all participants will be health screened, in case of heart disease history, blood pressure will be taken and anyone deemed to be hypertensive will be excluded from testing. Heart rate monitors will also be worn as a precautionary measure. Bruising can occur when the cuff is inflated to a high pressure however this study will be using a low pressure (80mmHg). It can be noted that current literature deems this exercise modality as safe. During exposure the researcher will monitor the participant's perceived pain, if the pain levels are too high the participant can withdraw from the study.

2. Any heat transfer from infra-red light will be negated due to the flow of blood, all equipment will be serviced with up to date PAT certificates. The NIRS device will also allow the constant monitoring of muscle oxygen levels, thus acting as a secondary safety mechanism whereby the researcher can terminate the exercise if this level drops dramatically.

3. Acute Mountain Sickness at heights of 3500 meters has a prevalence of 18% within the human population. AMS symptoms include; headaches, anorexia, insomnia, vomiting, shortness of breath, ataxia, Tachypnea, pulmonary rales/cracking; both periorbital and peripheral oedema. Testing will cease if participants experience any of these symptoms. Heart rates will be monitored constantly pre, during and after exercise until the participant has been removed from the testing area. A pulse oximeter will be used to monitor the contralateral arm throughout to prevent dangerously low levels of blood oxygen saturation (SpO^2), with a cut of limit of 80%. SpO^2. Participants will be informed of what is to be expected, and will also have the opportunity to familiarize themselves with the feelings associated with the changes during the rest period prior to exercise commencing.

4. Maximal contractions will occur pre cuff inflation to prevent muscle/ vascular damage associated with high intensity contractions under pressure. Prior to exercise a physical activity readiness questionnaire will be filled out to ensure you are healthy enough to exercise.

5. Blood screening forms will be filled out to prevent the transfer of blood born infections and diseases. To prevent infections, site of extraction will be cleaned with alcoholic wipes, protective the researcher will wear clothing, and all cuts or abrasions will be covered. During extraction participants will be sat or lying down to prevent falling in the event of feinting. The blood withdrawal will be performed by a trained phlebotomist.

What if you decide you want to withdraw from the project?

If, at any stage you wish to leave the project, then you can. There is no problem should you wish to stop taking part and it is entirely up to you. There will be no disadvantage to yourself should you wish to withdraw.

What will happen to the data and information collected?

Everyone that takes part in the study will receive their own results for the tests that they complete. All information and results collected will be held securely at the University of Bedfordshire and will only be accessible to related University staff. Results of this project may be published, but any data included will in no way be linked to any specific participant. Your anonymity will be preserved.

What if I have any questions?

Questions are always welcome and you should feel free to ask the lead researcher or project supervisor.

Should you wish to participate in this study then please complete the attached consent form, which needs to be returned before commencing the study.

This project has been reviewed and approved by the Ethics Committee of the Department of Sport and Exercise Sciences at the University of Bedfordshire.

Many Thanks,

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Appendix B

Consent Form

TO BE COMPLETED BY PARTICIPANT

Name of participant:…… Date:……

I have read the information sheet concerning this project and understand what is required of me as a participant. All my further questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:

- My participation in the project is entirely voluntary and I am free to withdraw from the project at any time without disadvantage or prejudice.
- I will be required to attend 5 sessions to complete the project.
- **As part of the study I will have to:**
- Adhere to pre-test exclusion criteria.
- Take part in maximal and mild intensity handgrip exercise.
- Have blood flow restricted during rest and handgrip exercise.
- Be exposed to hypoxia (low oxygen levels) during rest and handgrip exercise.
- Have blood withdrawn twice per a session by a trained phlebotomist.
- Have oxygen content of the exercising muscle monitored by an infrared device.
- **I am aware of any risks that may be involved with the project**.
- All information and data collected will be held securely at the University indefinitely.
- The results of the study may be published but my anonymity will be preserved.

Signature:……(Participant) **Date:** ……

Appendix C

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

Appendix D

BLOOD ANALYSIS – Participant Screening Form

Please read the following:

a. Are you suffering from any known active, serious infection?
b. Have you had jaundice within the previous year?
c. Have you ever had any form of hepatitis?
d. Have you any reason to think you are HIV positive?
e. Have you ever been involved in intravenous drug use?
f. Are you a haemophiliac?
g. Is there any other reason you are aware of why taking blood might be hazardous to your health?
h. Is there any other reason you are aware of why taking your blood might be hazardous to the health of the technician?

Can you answer **Yes** to any of questions a-g? Please tick your response.

Yes ☐ No ☐

Small samples of your blood (from finger or earlobe) will be taken in the manner outlined to you by the qualified laboratory technician. All relevant safety procedures will be strictly adhered to during all testing procedures (as specified in the Risk Assessment document available for inspection in the laboratory).

I declare that this information is correct, and is for the sole purpose of giving the tester guidance as to my suitability for the test.

Signed ……

Date ……

If there is any change in the circumstances outlined above, it is your responsibility to tell the person administering the test immediately.

The completed Medical Questionnaire (Par Q) and this Blood Sampling Form will be held in a locked filing cabinet in the School of PE and Sport Sciences laboratories at the University for a period of one-three years. After that time all documentation will be destroyed by shredding.

If you wish to have a photocopy of any of the completed documents, please ask for one.

Appendix E

Pre Exercise Study Preparation

Prior to your attendance to take part in the exercise study, please ensure you adhere to the following:

- Please do not partake in strenuous exercise 72 hours prior to participation in this exercise study or during.

- Please do not consume alcohol or caffeine 72 hours prior to participation in this exercise study or during.

- 30 days leading up to the exercise study and during please ensure you have not had exposure to the following:

- Dietary or vitamin supplementation in the form of: anti-oxidants, nitrates, protein and branch chain amino acids.

- Ergogenic aides/performance enhancers: L-Arginine, β- Alanine and creatine.

- Exposure to extreme environments: hypoxia (low oxygen), hyperthermia (heat) and hypothermia (cold)

I can honestly say that I have adhered to the above criteria leading up to my participation in the current exercise study and sign below to confirm this:

Familiarisation Session One:

…….

Familiarisation Session Two:

…….

Exercise Session One:

…….

Exercise Session Two:

…….

Exercise Session Three